THE MECHANISM OF FLIGHT PREPARATION IN SOME INSECTS*

BY AUGUST KROGH AND ERIK ZEUTHEN
From the Laboratory of Zoophysiology, University of Copenhagen

(*Received* 5 *February* 1940)

(With Eight Text-figures)

928 Dotterweich published under the title "Beiträge zur Nervenphysiologie der kten" some very interesting and important observations and measurements ly on Sphingidae, showing that the rapid vibration of the wings regularly rved in large moths before they take flight produces an increase in temperature e wing muscles which is necessary to enable them to fly at all. The temperature ned was nearly the same in all cases, 32–34° C., and the time taken to reach it d with the initial temperature deficit, up to 6 min. with a deficit of 23°. Moths at 34° or above would fly off at once, without any preparation, when stimu-. It had been assumed by v. Buddenbrock that this preparation was mainly red to attain the high frequency of the wing movements necessary for actual t, but a number of observations made by Dotterweich on butterflies (*Vanessa*), hich the normal frequency is much lower, made it very probable that in these preparatory wing movements were responsible for a rise in temperature ssary for actual flight.

The general interest of these observations became greatly enhanced when it was n by Marius Nielsen (1938) in this laboratory that, in man, muscular work is ly correlated with a rise in body temperature which is regulated to very ly defined higher levels corresponding closely to the rate of work.

The present paper contains a confirmation and extension of Dotterweich's ts. Temperature measurements have been made throughout by means of mojunctions made of 0·1 mm. constantan and 0·2 cm. copper wires inserted ugh the integument of the insect in question. In most cases the wires ending e thermojunction had a vertical free length of 30–40 cm. allowing the insect a iderable amount of freedom to move, while flight could be induced, in certain itions, by lifting the insect from the support (Fraenkel, 1932).

THE TEMPERATURE OF INSECTS DURING REST

achmetjew (1901), Necheles (1924) and others have shown and we have con-d that during rest the temperature of insects is not very different from that of environment, being in moist air slightly higher and in fairly dry air often itely lower on account of the evaporation of water.

*Owing to war conditions, the authors have been unable to submit a corrected proof prior to cation.

We found, for instance, on a bumble bee weighing 200 mg. the results shown i Table I, and in a dor-beetle, *Geotrupes stercorarius*, weighing 900 mg. in dry air th figures given in Table II.

Table I

In saturated air			In dry air		
Temperature of			Temperature of		
Chamber ° C.	Thorax ° C.	Diff. ° C.	Chamber ° C.	Thorax ° C.	Diff. ° C.
26·8	27·4	+0·6	14·4	14·1	−0·3
			20·5	20·0	−0·5
			28·3	27·6	−0·7
			31·8	31·2	−0·6
36·1	37·2	+1·1	35·9	35·1	−0·8
			39·9	38·9	−1·0
			42·2	41·2	−1·0

Table II

Chamber ° C.	Thorax ° C.	Diff. ° C.
12·1	12·2	+0·1
23·1	23·0	−0·1
29·9	28·8	−1·1
37·1	35·1	−2·0
42·6	40·1	−2·5

In order to raise the temperature significantly above that of the environmen the metabolism must be considerably increased, and this is done and can apparentl only be done by muscular activity.

THE PREPARATION FOR FLIGHT IN BUTTERFLIES (*VANESSA*)

It is a common observation that butterflies, which at a medium or rather lo temperature can be quite active so long as they can absorb a great deal of radian heat from the sunshine, will come to rest almost immediately when a cloud passe In this state they are not capable of instant flight, but, as Dotterweich has describe and figured, flight is preceded by a period of preparation during which the win move. Dotterweich distinguishes between two main types of such movements. I the "pumping" type the wings are moved at a not very rapid rate (1 per sec through an angle of about 40° from the horizontal. In the vibratory movemen ("Schwirren") the movements are much more rapid, but the angle quite smal Vibration takes place usually about the resting position with wings almost vertica but may also occur in wings spread out almost horizontally. We have observed th same types as Dotterweich and find most often the vertical vibration with a fr quency of about 15 per sec. as judged from the sound produced.

In the typical experiment recorded in Fig. 1 a thermojunction was inserted into ne wing muscles of a *Vanessa atalanta* and the temperature read at frequent ntervals. The animal was suspended by the thermojunction wires and, sometimes

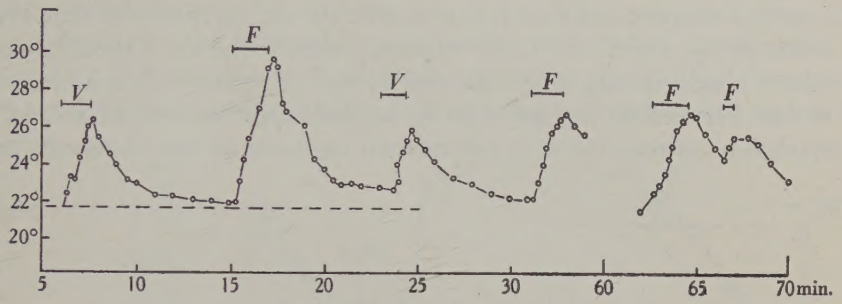


Fig. 1. Temperature record of *Vanessa*. *V*, vibration of wings; *F*, flight; --- air temperature.

pontaneously, sometimes after a slight mechanical stimulus, either flew or heated self by wing vibrations. In the first period wing vibration caused a rapid rise in emperature in the animal which was otherwise quiet and did not attempt to fly. n the second period flight was induced by stimulation and lasted for about 2 min. uring which the temperature rose from 22 to 29°.

In another individual (Fig. 2) flight began spontaneously when a tempera- ure of 35° had been reached by vibration. Generally we find that flight is possible in utterflies at all temperatures above 20°, but sually the animals will raise their temperature y vibration before flight, or alternatively uring the first minutes of flight the tempera- ure is seen to increase rapidly. During this ame period the flight is intensified, the animal fting itself and the suspending wires higher nd higher and the angle through which each ing is beating increasing from 120° to nearly 80°.

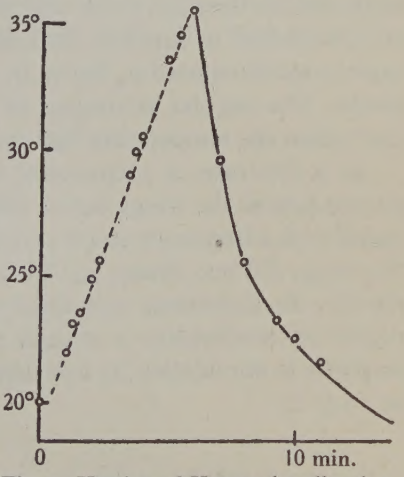


Fig. 2. Heating of *Vanessa* by vibration, with subsequent cooling.

We cannot subscribe to Dotterweich's tatement that moths require a definite emperature to be able to fly at all. We made few observations on *Catocala sponsa*, neasuring thoracic temperature and then hrowing the moth into the air. These observations indicate that at muscle emperatures above 25° this species is able to fly.

OBSERVATIONS ON BUMBLE BEES, *BOMBUS HORTI*

In several cases the thoracic temperature of a bumble bee was measured, the thermojunction removed and the bee thrown into the air. At measured temperatures below about 30° the insect was unable to sustain itself in the air. Thoracic temperatures in bees caught during flight and rapidly put on to the thermojunction were found to vary between 37 and 42° with air temperatures between 22 and 32°. No clear correlation between thoracic and external temperature was observed, but the

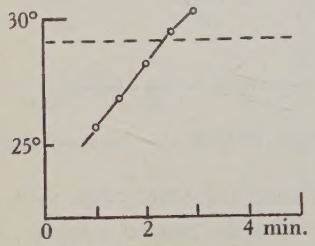


Fig. 3. Heating by flight movements of bumble bee.

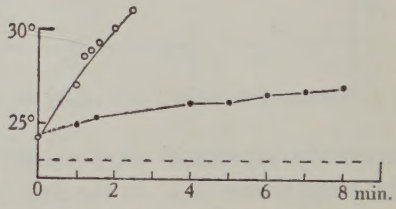


Fig. 4. Thoracic (o) and abdominal (•) temperatures of bumble bee during process of heating.

abdominal temperature measured in a few cases was clearly influenced by the environmental temperature which it exceeded by 2–5°. The inability to fly at lower temperatures does not mean that no flight movements are performed, but that they are insufficient to support the animal in the air. This is well brought out in the experiment illustrated in Fig. 3, in which the insect "flew" while suspended on the needle. The angular excursions of the wings increased from about 30° at 25° C. to 120° when the temperature had reached 30° C.

In a few cases a preparation for flight was observed, consisting in vibratory movements of the wings before they were spread for flight. This produced a loud sound with a frequency above 100 per sec., localized in the muscles, since removal of the wings did not change it. During such vibration the thoracic temperature rose rapidly, the abdominal only slowly (Fig. 4). It is worthy of note that this thermoregulatory mechanism is utilized not only as a preparation for flight, but also in response to stimulation by low temperature when the insects were transferred from 20 to 5° C.

EXPERIMENTS ON THE DOR-BEETLE (*GEOTRUPES STERCORARIUS*)

While the butterflies, moths and bumble bees rank as fairly good fliers, capable of varying the rate of flight within wide limits, the flying power of the lamellicorn beetles is limited and they can vary the speed only within a narrow range. It is well known that they have normally to "pump" for a certain length of time before flight. This "pumping" is a series of deep and frequent abdominal respiratory movements. It was very generally assumed formerly that the pumping served to fill up the tracheal system with air or to raise the concentration of oxygen, but Dotter

reich suggested that it was correlated with a process of heating, necessary for iight. There is the difficulty, however, that no wing movements are visible during pumping", and it is scarcely to be imagined that the respiratory movements nemselves could liberate sufficient energy.

A dor-beetle can be induced to prepare for flight when placed on a small latform like a cork from which it is difficult to climb down, provided the conditions f light and temperature are suitable. In the typical experiment recorded in Fig. 5

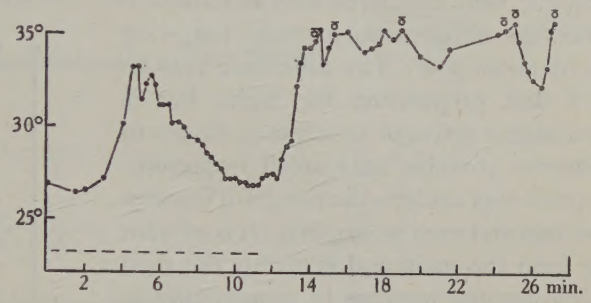


Fig. 5. Temperature record of *Geotrupes*. ō, attempts to fly.

ne thermojunction was introduced through the scutellum into the wing muscles f the mesothorax. From the 2nd to the 5th minute the animal "pumped" but iid not attempt to fly and cooled off again. Later, the elytra and wings were pread repeatedly and abortive attempts at flight were made, but were promptly

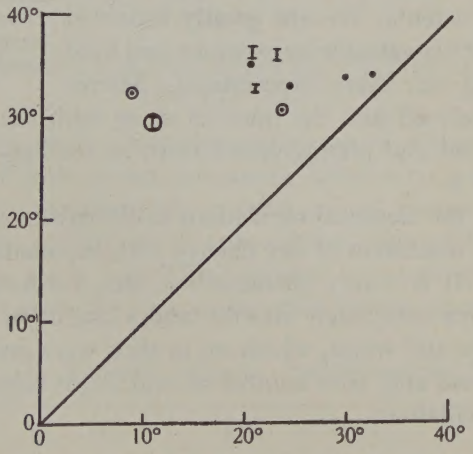


Fig. 6. Relation between thoracic and environmental temperature in *Geotrupes* at beginning of flight. I, repeated attempts. • unsuccessful attempts.

nhibited by the restraining influence of the wires. All the experiments on this pecimen agree in indicating a temperature of $35° \pm 0{\cdot}5$ in the wing muscles just efore flight, but from one animal to another the flight temperature may vary etween 32 and 37°. Attempts occasionally made at lower temperatures were un-uccessful. As shown in Fig. 6 the thoracic temperature when flight begins is

practically independent of the environmental temperature. The animals attempti flight at about 30° thoracic temperature did not succeed. In these cases the frequen of the wing beats appeared normal, but the amplitude was much reduced. Ev when not restrained by any instrument certain specimens would attempt flight aft insufficient preparation at environmental temperatures of 11, 20–22 and even 3 and tumble down.

In the following experiments temperatures in the mesothorax containing t principal wing muscles were compared with abdominal and prothoracic. In all cas it was found that the temperature outside the wing muscles is lower by about 5–6°. The difference may be even larger in a first preparation for flight, but is gradually reduced after a series of attempts as shown in Fig. 7. The abdomen contains only small respiratory muscles, but the prothorax contains the powerful muscles supplying the forelegs and used in digging. It is evident therefore that at least the main and probably the total activity responsible for the increase in temperature is confined to the wing muscles.

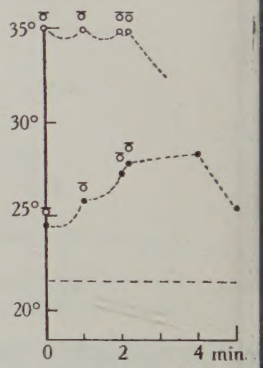


Fig. 7. Prothoracic (•) a mesothoracic (o) temperatu of *Geotrupes* attempting fly (ō). Prothoracic tempe tures were actually measur mesothoracic inferred from similar experiment on the sa specimen.

It is a very curious fact that this activity does not cause any visible movements of the wings as is ascertained by observations with a lens after complete removal of the elytra. That the activity does nevertheless take the form of muscular contractions has been shown by recording the action currents. We are greatly indebted to Dr F. Buchthal for his valuable suggestions and kind assistance in carrying out these experiments. Micro-electrodes were introduced into the muscles along with the thermojunction, a the potentials amplified and photographed from an oscillograph. A typical cur is reproduced in Fig. 8.

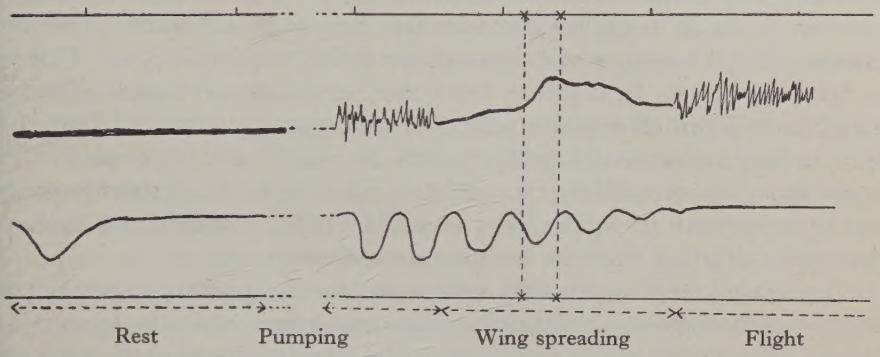
The frequency of the electrical oscillations is difficult to estimate (about 60 p sec.), but there is no indication of any change with increasing temperature with the interval 24–35°. It is a very characteristic fact, verified repeatedly, that t muscular activity stops completely shortly before the flight begins. Less than second later the elytra and wings, which up to then were only slightly lifted fro the abdomen, are spread and, after another second, flight begins, showing of cou renewed electrical oscillations.

THE VENTILATION OF THE TRACHEAL SYSTEM

During rest (at about 22°) the respiratory movements show a frequency of o 20–25 per min. They are confined to the anterior part of the abdomen and can conveniently observed only after removal of the elytra and wings. In preparati for flight the frequency is at once increased to between 180 and 240 per min., wh the depth is increased gradually until involving the whole of the abdomen, and at t

me time the elytra are lifted and spread slightly. During the pause in muscular tivity intervening between preparation and actual flight there is a definite decrease respiratory amplitude, and during the initial stages of flight no abdominal spiration can be observed as noted by Fraenkel (1932).

A diagram of this course of events is included in Fig. 8.



. 8. Oscillogram of wing muscles and rhythm of respiration in *Geotrupes* preparing for flight. per line time in seconds. The action potentials cease 1½ sec. before the flight. The spreading of the gs causes a displacement of the electrode. The respirations are reduced during this period and se when flight begins.

Like the bumble bee the dor-beetle is sometimes seen to react to a cold environ-nt by heat production in the wing muscles. In these cases the oscillogram dicated a lower frequency, e.g. 7–14 per sec. at 11° body temperature.

THE HEAT PRODUCTION INCIDENTAL TO THE PREPARATION FOR FLIGHT

From the curve (Fig. 2) the increase in temperature of the wing muscles of the tterfly can be read off from minute to minute. When after 6 min. the animal took ght this was immediately inhibited, and the animal cooled down to the initial mperature. From the cooling curve approximate rates of heat loss during the ating process can be made out. These figures are put together in Table III, s. 1 and 2, and col. 3 gives the approximate temperature increment corrected for

Table III. Vanessa atalanta. *Preparation for flight*

Min.	(1) Temperature increment °C.	(2) Estimated loss °C.	(3) (1)+(2) °C.	(4) (3) × 0·8 heat produced cal./g./min.	(5) Whole animal Cal./kg./hr.	(6) Whole animal l. O_2/kg.h.
1	20 –22	0·5	2·5	2	40	8
2	22 –24·6	1·5	4·1	3·3	66	13
3	24·6–27·5	2·5	5·4	4·3	86	17
4	27·5–30·2	4	6·7	5·35	107	21
5	30·2–33·0	5	7·8	6·25	125	25
6	33·0–33·5	7	9·5	7·6	152	30

heat loss. The wing muscles in which the heat is produced make up a little over one-fifth of the total body and we assume the total mass of substance heated to the temperature measured to make up one-third of the body weight and to have a heat capacity of 0·8 %, the normal value for animal tissues. Making these assumptions we have the total heat production in cal./g./min. of the active tissue (col. 4) and the corresponding heat production for the whole animal given in cal./kg./hr. (col. 5). As the butterfly feeds on sugar we can take this heat to result from carbohydrate combustion, using 1 l. oxygen and eliminating 1 l. CO_2 to produce 5 cal. This gives us the figures in col. 6. In this case direct determinations were made of the CO_2 eliminated during two consecutive periods from 0·5 to 2·3 min. and from 2·3 to 4·7 min. corresponding to the temperatures 20·9–25·6° and 25·6–32·1°. These determinations gave respectively 0·12 and 0·24 ml. CO_2 for the butterfly weighing 320 mg. or, calculated per kg./hr., 12·5 and 18·8 l. CO_2, a satisfactory agreement with the values deduced from the temperature measurements.

Similar calculations of metabolism were made from temperature curves obtained on *Geotrupes* indicating only slightly lower maximum values (26 l. CO_2/kg./hr.), but a more rapid rise with increasing temperature. In actual flight much higher rates of metabolism can be reached.

DISCUSSION

While the possibility that the heating process described is essential for the discharges of nervous impulses from the corresponding ganglion to the muscles cannot be definitely excluded, it appears much more likely that it is required to allow the muscular engine to develop the energy required for flight.

If the mechanism had to do primarily with the nervous discharges one would expect the frequency to be mainly involved, but this is certainly not the case, as shown by the fact that the heating vibrations have a similar frequency as, but a much smaller amplitude than, the regular flight movements, a frequency which is probably less than 20 per sec. in butterflies and more than 100 per sec. in bumble bees.

Accepting the heating as essential for the muscular production of energy we may attempt a rather wide generalization. It is well known that the minimum or "standard" metabolism of animals during rest is governed by body temperature and can be expressed as a definite function of temperature, the same for quite a number of forms, different in others. The present temperature measurements (and a number of observations of animal behaviour) suggest an equally definite relationship between the possible maximum muscular activity and the temperature of the working muscles. It should be emphasized, however, that at present we cannot get beyond the suggestion of a relationship; to prove its existence or to obtain a quantitative formulation will be a very difficult task.

The need for "heating up the engine" before flight is no doubt widespread among insects, but by no means general. We believe it to exist in all "bad" fliers who require a maximal or nearly maximal muscular effort to fly at all, as is the case

th many beetles. It is probably significant that several species of *Hydrometra* are ngless near the northern boundaries for their distribution, but farther south are nged and able to fly. The good fliers, especially among the Diptera, are able to fly at ther low temperatures, but attain to high velocities only when the environmental mperature is fairly high, as it can be easily observed on the common housefly. It scarcely conceivable that small insects like mosquitos are able to raise the mperature of their wing muscles more than a couple of degrees above the en- ronment, but nevertheless *Trichocera hiemalis* can be observed flying in winter at mperatures about the freezing point.

The line of investigation initiated in this paper will be followed up in two stinct directions. One of us (E. Z.), who began these experiments, is mainly terested in the problems of heat regulation. It is evident that, when during flight e metabolism is further greatly increased, mechanisms must come into play which ll prevent any increase in temperature beyond a certain point, and it is suggested at a regulation of evaporation is responsible. The senior author has been interested r years in problems concerning economy of locomotion and proposes to study the etabolism of insects during flight at various known velocities, and to discover if ssible, the influence of temperature upon the maximal rate of flight.

SUMMARY

Temperatures have been measured by means of thermojunctions at several ints in the body of insects preparing for flight.

In butterflies (*Vanessa*), moths and bumble bees (*Bombus*), preparation consists vibratory movements of the wings raising the temperature of the wing muscles ually above 30° C. In lamellicorn beetles (*Geotrupes*) there are no visible move- ents of the wings, but the vibrations can be demonstrated by leading off action tentials from the muscles. The heat production takes place always in the wing uscles, but there is a gradual and much slower increase in the temperature of the st of the body.

The muscle temperature necessary for flight is high (above 32°) and nearly nstant in bad fliers (*Geotrupes*), while the good fliers can fly at different tempera- res, *Vanessa* from about 20 up to 42°. Even in the latter type a high rate of flight n be attained only when the wing muscles have become heated above 35°.

The heat production in the vibrating muscles can be estimated from the mperature increment during the heating process combined with the decrement ring subsequent cooling. It increases rapidly with increasing temperature. The al value found in *Vanessa* just before flight at 34° corresponds to a metabolism 30 l. O_2/kg./hr. The metabolism in actual flight reaches much higher values.

It is suggested that a definite relationship may exist between temperature and e maximum work of which a muscle is capable.

Our thanks are due to Dr F. Buchthal for his valuable suggestions and kind lp to obtain oscillograph records of muscular vibrations.

REFERENCES

BACHMETJEW, P. (1901). *Experimentelle entomologische Studien vom physikalisch-chemischen Standpunkt*, **1**. Leipzig.

DOTTERWEICH, K. (1928). *Zool. Jb., Abt. allg. Zool. Physiol. Tiere*, **44**, 399–425.

FRAENKEL, G. (1932). *Z. vergl. Physiol.* **16**, 394–417.

NECHELES, H. (1924). *Pflüg. Arch. ges. Physiol.* **204**, 72–86.

NIELSEN, M. (1938). *Skand. Arch. Physiol.* **79**, 193–230.

OVULATION AND OVIPOSITION IN ANURA

By H. WARING, F. W. LANDGREBE
and R. M. NEILL

From the Department of Natural History, University of Aberdeen

(*Received* 1 *October* 1940)

(With Three Text-figures)

INTRODUCTION

results of hypophysectomy and of injection of extracts show that the gonads of phibia are under the control of the anterior lobe of the pituitary (Wolfe, 1929; ben, 1930). *Xenopus laevis* does not exhibit any sexual activity in the laboratory. n appropriate nutritive and other conditions are fulfilled (Landgrebe, 1939), the ies are close to the threshold of ovulation at all seasons. Injection of gonado- ic extract then leads to ovulation and oviposition. If males in the same container ive the same treatment, clasping occurs and fertilized eggs result (Shapiro, 1936). ature *Xenopus* does not shed simultaneously all the eggs which ripen in a season. habits of ovulation and oviposition are essentially similar to those of Urodeles. *a*, on the other hand, sheds simultaneously at the breeding season all the eggs ch have ripened during the preceding year, and there is a rapid growth of oocytes ch have not previously attained visible dimensions about the time when ovulation ns. Injection of gonadotropic extracts causes hypertrophy of the ovaries and lation but rarely oviposition (Bellerby, 1933).

There are other differences in oviposition between *Xenopus* and *Rana*. The eggs *Rana* are shed into the body cavity. They pass into and down the upper part of oviduct by ciliary action (Rugh, 1935). Then they descend to the lower third of duct ("uterus") and are retained there for days or weeks. Eventually they are elled *en masse*. The oviduct is hypertrophied in the sexual season and from its the gelatinous egg envelope is formed (Rugh, 1938; Marsh, 1938). Marsh has wn that oviducts become hypertrophied by injection of extracts of the anterior e of the pituitary (A.L.P.) combined with oestrin. This does not necessarily n that the normal enlargement of the ducts is due to the combined effect of e two autocoids, since there is as yet no evidence for the production of oestrin in phibia.

In South African ponds *Xenopus* lays its eggs singly with a large amount of jelly. hough the eggs which *Xenopus* can be induced to lay by administration of A.L.P. fertile, such treatment always results in the extrusion of eggs with little jelly. s is probably because the oviducts are permanently small in the laboratory. That ry action is sufficient to bring about oviposition after ovulation in *Xenopus* can lemonstrated by inserting eggs into the body cavity through an incision which

is then stitched up (sealed). Such eggs are passed out into the surrounding medium in a few hours. The same procedure does not result in complete extrusion of the eggs of *Rana*; these pass down the upper part of the oviduct for some distance and then stop.

Cilia can be detected down the whole length of the oviduct of *Xenopus*. We have been unable to detect them in the lower third of the oviduct in *Rana*, even when examination is made shortly after oviposition. Observations were made at this time because it is not intrinsically improbable that there is a seasonal variation in the development of cilia under the influence of sex hormones, e.g. the development of cilia in the mammalian vas deferens after injection of androgens (Vatna, 1930). In any case it is unlikely that ciliary action could move the large mass of eggs which accumulate in the greatly distended lower part of the oviduct of *Rana* prior to oviposition.

Thus ovulation in *Xenopus* is always followed by oviposition. In *Rana* ovulation and oviposition are two entirely separate problems. The agencies responsible for ovulation and for passage of the eggs down the top part of the oviduct are known, Rugh (1938) does not describe their passage further in the normal animal. Hitherto the ultimate extrusion of the egg mass from the lower part of the oviduct (other than by "stripping") has not been investigated.

In elasmobranchs, reptiles, birds and monotremes the complete sequence of female reproductive activity includes the following physiologically distinct events each of which presupposes its appropriate antecedent stimulus:

(1) ripening of the ovarian egg;
(2) liberation of ovarian egg;
(3) the migration of liberated egg from ovary to the oviduct;
(4) movement of the egg down the upper part of the oviduct;
(5) secretion of an albuminous coat;
(6) secretion of shell membrane and shell;
(7) extrusion of the completed egg.

In Anura the mechanism of stages 1–3 is already known (Rugh, 1938). It is well established that in the intact animal both 1 and 2 are controlled by the anterior lobe of the pituitary gland. We have made experiments to test the action of other gonadotropic substances on the ovary of *Xenopus* in view of recent work in this field.

Stage 4 is, as has been already pointed out, due to the action of the ciliated lining of the oviduct. In *Xenopus*, but not in *Rana*, no distinction need be made between 4 and 7. Stage 6 is clearly lacking in Amphibia.

On the assumption that stage 5 is represented by the secretion of the egg jelly in Amphibia, there remain therefore, two outstanding problems of the Anuran reproductive cycle in regard to which information is lacking, i.e. the nature of the effective stimulus to, and co-ordinating mechanism which controls (*a*) the secretion of egg jelly, (*b*) the expulsion of the ripe egg from the lower part of the oviduct in the Phaneroglossa (e.g. *Rana*).

I. THE EFFECT OF VARIOUS GONADOTROPIC SUBSTANCES ON THE OVARY OF *XENOPUS*

Removal of the anterior pituitary leads to genital atrophy in *Rana* and in *nopus*. Injection of anterior lobe extracts evokes enlargement of the ovary and ulation. There is therefore complete justification for the statement (p. 11) that anterior pituitary controls the cyclical variations of the ovary.

The gonadotropin present in pregnancy urine also evokes ovulation in *Xenopus* llerby, 1934) and has been made the basis of a pregnancy diagnosis method ndgrebe, 1939; Crew, 1939). Recent work in the field of pregnancy diagnosis, which specificity is of paramount importance, has focused attention on the fact t substances other than the generally recognized gonadotropins—A.L.P. and P.U. will evoke ovulation.

In view of current controversy on the existence or otherwise of two classes gonadotropins with different properties—one follicle stimulating, the other einizing—experiments were made to test the effect of various substances on the ary of *Xenopus*.

Progesterone and other steroids (Shapiro & Zwarenstein, 1937) induce ovulation d oviposition in *Xenopus*. Other workers have not recorded whether these subnces have a direct effect on the ovary. Certainly Shapiro & Zwarenstein (1937) orded ovulation in the excised ovary of *Xenopus* immersed in frog Ringer after lition of progesterone and other substances: in our experience, however, heart fusion experiments have shown that frog Ringer is an unsuitable medium for *nopus* tissues. We therefore tested the effect on the intact animal.

Table 1 which records observations on *Xenopus* after anterior lobe removal ows that the immediate stimulus to ovulation is direct. The doses given were: .c. pregnancy urine, 2 mg. progesterone, 1 g. anterior lobe tissue. We have not de experiments to find whether the *reduced* ovary of a hypophysectomized animal affected by these substances.

Table 1

No. in each group	No. of days between operation and injection	No. ovulated		
		A.L.P.	P.U.	Progesterone
6	1	6	6	4
6	8	6	5	3

Zondek (1930) observed that urine of castrate women (or menopausal urine) en injected into intact mice produced a predominantly follicle stimulating effect. jections of pregnancy urine had a predominantly luteinizing effect. At that time ndek considered that the gonadotropic substances of both these fluids originated the pituitary and he called that from menopausal urine Prolan A (follicle stimuing) and the luteinizing substance Prolan B. He believed that pregnancy urine ntained some A as well as B. There is now good reason to believe that Prolan B chorionic in origin.

Fevold (1939) prepared two fractions from ox anterior lobe which he called follicle stimulating hormone and luteal hormone. In their effect on *intact* animals the former (F.S.H.) is roughly equivalent to Prolan A and the latter (L.H.) to Prolan B.

Maxwell (1934) and Saunders & Cole (1936) have shown that certain gonadotropically inert substances such as casein and zinc sulphate when injected at the same site as pituitary extract augment its effect. Such substances do not have this effect when injected at a different site from the pituitary extract. Zinc sulphate is believed to act on the process of absorption of the gonadotropin. Its effect is not to be confused with that of substances such as picric salts (Marshall *et al.* 1939) which when injected alone into intact rabbits induce ovulation by stimulating the intact pituitary.

Some attempt has been made to align the pituitary fraction L.H. with inert substances such as zinc sulphate and to explain its action as merely increasing the effect of one potent gonadotropin (F.S.H.). It has been pointed out that a definite criterion of a true L.H., as distinct from an inert substance which locally facilitates absorption of F.S.H., is to prove its efficacy when injected at a separate site from the F.S.H. A more satisfying test, however, is to demonstrate that the alleged F.S.H. will produce enlarged follicles but no lutea when injected in excess into hypophysectomized rats and that L.H. is without effect on the reduced ovary of hypophysectomized rats but causes luteinizing when injected after the follicles have been enlarged by previous injections of F.S.H. This has recently been accomplished (Fevold; 1939). Quite apart from this convincing demonstration it is now generally agreed (Summary, Liu & Noble, 1939) that pregnancy urine, although having a generalized gonadotropic effect on intact animals produces no follicular maturation in the reduced ovary of hypophysectomized rats.

The following experiments were performed to determine whether pregnancy urine evoked any response in the reduced ovaries of hypophysectomized *Xenopus*. The ovaries of six hypophysectomized *Xenopus* were examined under anaesthesia. They were all atrophied. The animals were placed in separate containers and two injections of 3 c.c. pregnancy urine were administered each week for several weeks. All the animals ovulated.

If we are prepared to believe that there are two separate gonadotropins which produce distinct effects on the mammalian ovary, such a mechanism is clearly a late evolutionary development.

II. SECRETION OF EGG JELLY

In *Rana* egg jelly is secreted by the epithelium of the oviduct which is enlarged and glandular at the breeding season. Enlargement of the oviduct may be elicited out of season by the injection of oestrin and A.L.P. (Marsh, 1938).

Xenopus eggs produced after injection of gonadotropin in the laboratory are covered with much less jelly than are those eggs which occur naturally in South African ponds. This may be due to the fact that in nature *Xenopus* sheds its eggs slowly, one at a time, while after injection up to 5000 eggs are shed in a few hours. Such eggs are fertile, develop and the larvae metamorphose apparently quite

mally. Naturally occurring enlargement of the oviduct of wild *Xenopus* ring the breeding season is not familiar to us, but the oviducts after injection er reach a size comparable to that well known in *Rana* at the breeding son.

An attempt was made to obtain a larger gelatinous envelope in *Xenopus* by giving ial injections of A.L.P. on the working assumption that the oviduct has a lower eshold to stimulation than the ovary and that gradually increasing pituitary levels ght allow time for the oviducts to enlarge before the threshold of ovulation was ched. Twelve *Xenopus* were selected from a batch which consistently responded ·5 g. of fresh anterior lobe tissue. They were injected daily with the equivalent ·1 g. of fresh anterior lobe tissue. On the fifth day seven of the twelve had shed w eggs and all twelve had ovulated by the seventh day. While demonstrating the litive effect of continued low doses this experiment did not produce more jelly n is usual after one injection. It remains a possibility that injections spread over nger period might have the desired effect.

Extracts of the posterior lobe of the pituitary injected together with an extract he anterior lobe had no effect on the number of *Xenopus* which ovulated (Table 2) l had no effect on the amount of jelly secreted.

Table 2

Anterior lobe + muscle extract						Anterior lobe + posterior lobe					
No. of animals injected	Dose in g.		No. ovulated in			No. of animals injected	Dose in g.		No. ovulated in		
	Ant.	Muscle	18 hr.	24 hr.	48 hr.		Ant.	Post.	18 hr.	24 hr.	48 hr.
12	½	¼	12	12	12	12	½	¼	12	—	—
11	¼	¼	10	10	10	11	¼	¼	8	9	10
12	¼	½	5	9	12	12	¼	½	6	9	11

Single injections of either pilocarpine, adrenalin, pitocin, oestrin or posterior e extract, made at the same time as injection of anterior lobe extract, had no ct either on the number of animals responding or on the gelatinous envelope.

Six *Xenopus* were injected with 0·01 mg. of oestrone twice daily for 3 days. ereafter the animals received 0·2 g. fresh anterior lobe tissue daily. All the mals ovulated by the seventh day but with no increase in the amount of jelly reted.

Table 3

Group	Av. weight of 3 animals g.	Av. weight of ovary g.	Av. weight of oviduct g.	Oviduct body weight g.
Control	40·0	4·5	0·83	0·21
3 months after hypophysectomy	36·3	1·6	0·46	0·13
3 months after ovariectomy	34·6	—	0·48	0·14

Failure to obtain an increase in the secretion of egg jelly led the authors to investigate gravimetrically induced changes in the oviduct. Removal of the pituitary in *Xenopus* results in atrophy of the ovary and of oviduct within a few months. Ovariectomy also results in atrophy of the oviduct (Table 3). So far we have been unsuccessful in obtaining a *Xenopus* ovarian extract which will prevent atrophy of the oviduct after hypophysectomy.

III. THE MECHANISM OF EXTRUSION OF RIPE EGGS IN *RANA*

It was first determined whether the presence of a clasping male is necessary for oviposition. Thirty-two couples were separated. Oviposition occurred in twenty-eight of the females: four died with eggs *in utero*. During the same period fifty-one (100%) clasped females oviposited. The male is therefore not essential to oviposition. Two observations suggest that his presence is advantageous. In a number of the separated females extrusion was incomplete and in most of them oviposition was considerably delayed, as Rostand (1934) also found. Prima facie the male would appear at least to aid oviposition by direct mechanical pressure of the female body wall. In this connexion Smith's finding may be noted that while at coupling time there is no evidence for rhythmic movement of the pectoral muscles of the male, there is a great increase in the sensitivity of these muscles to faradic stimulation of the C.N.S. reaching its peak at the normal time of oviposition (Smith, 1938). Further, there is the possibility that older females may become conditioned to oviposit when clasped.

The following agencies are potentially involved in the extrusion of ripe eggs in *Rana*:

(1) The male, by direct mechanical pressure on the female body or otherwise. In a small proportion of cases gentle pressure applied to the body of gravid frogs results in egg extrusion.

(2) Ciliary action of the oviducal lining generally.

(3) Swelling of the ova and/or jelly coat due to imbibition of water, thus forcing the passage out through the utero-cloacal apertures.

(4) Muscular activity (*a*) of the uterus, analogous to mammalian parturition, (*b*) of the belly wall, (*c*) possibly in association with 4 (*a*) and/or 4 (*b*)—of other muscles of the posterior body region.

No. (1) will be discussed subsequently (p. 23).

It has been pointed out already that ciliary action (2) is inadequate to bring about extrusion of ova in *Rana*.

No. (3) becomes a real possibility when the size of the jelly envelope of eggs in the uterus is compared with that of eggs which have been deposited. To test this gravid females were kept in minimal humidity conditions. They laid eggs of the same size as those found *in utero*.

(4) *Muscular activity*

The relations of the oviducts to the cloaca and associated parts in *Xenopus* are n as to allow easy egress of ova transported singly down the oviducts by cilia and ted possibly with the additional aid of contraction of the abdominal muscles into ppropriate medium (water), irrespective of the time of ovulation. In *Rana*, on other hand, an Amphibian whose habits are largely terrestrial, the presence of r may or may not coincide with ovulation and passage of eggs into the oviducts. development of a collecting portion in the oviduct, i.e. "uterus," is consistent a lag between ovulation and actual oviposition. In these circumstances en- ed control of the actual emission of the ova at the cloacal end of the uterus is rly to be expected.

ndocrine control.

Mechanically two quite distinct events may be distinguished at parturition ome mammals. The uterus undergoes muscular contractions which tend to e the contents out, and evacuation is facilitated in some forms by the dis- ion of the distal passageway (vaginal region) as, for example, in the relaxation he pelvic ligaments that occurs in guinea-pigs during pregnancy (Hisaw, 1929), resorption of the pubic bones at the approach of puberty in the pocket gopher aw, 1925) and the loosening of the sacroiliac junction, and separation of the es at the symphysis which occasionally occurs during pregnancy in women ramson *et al.* 1934; Thoms, 1936).

Resorption of the pubic bones in the gopher is dependent on oestrin and the xation of the pelvic ligaments in the guinea-pig on a synergistic action of relaxin[1] oestrin (Hisaw, 1925, 1929). In view of these facts and since labour in mammals enerally believed to be initiated by a synergistic action of oestrin and oxytocin, first efforts were directed to removal of the ovary and pituitary and the injection ppropriate extracts. In this connexion it may be noted that the frog pituitary ds extracts with oxytocic properties (Hogben, 1923). The pituitaries were re- ed from fourteen gravid female frogs, the ovaries from fourteen others. Following operation the male was allowed to reclasp the female. Removal of both ovary pituitary was always fatal. The results are tabulated (p. 18).

These findings show that expulsion of eggs can take place in the absence of ian and pituitary secretions. It is improbable that effective quantities of these tances remain in circulation 2 or 3 weeks after extirpation of the glands. There o index of the level of ovarian hormones in the blood, but in mammals it is ainly excreted (or destroyed) fairly rapidly. Some idea of the amount of posterior hormones in the blood can be obtained by melanophore readings. The stimula- of the pituitary which immediately accompanies total hypophysectomy does not the melanophore index significantly. Injections of the oxytocic fraction of

[1] De Fremery *et al.* (1931) and Tapfer & Haslhofer (1935) have observed widening of the guinea- mphysis pubis after injection of oestrin and express doubt as to the existence of a water soluble hormone relaxin.

posterior pituitary extract either alone or following previous injections of oestrin were without effect on expulsion.

(*a*) Ovary removed 14	Died without oviposition 8	Oviposited 6 2 oviposited 1 day after operation 1 „ 4 days „ „ 1 „ 6 „ „ „ 1 „ 12 „ „ „ 1 „ 18 „ „ „
(*b*) Complete hypophysectomy 11	Died without oviposition 4	Oviposited 7 1 oviposited same night as operation 1 „ 3 days after operation 1 „ 4 „ „ „ 1 „ 8 „ „ „ 1 „ 12 „ „ „ 1 „ 15 „ „ „ 1 „ 23 „ „ „
(*c*) Anterior lobe extirpation 3	Died without oviposition 1	Oviposited 2 1 oviposited 4 days after operation 1 „ 17 „ „ „

That extrusion of eggs can take place in the absence of ovarian and pituitary secretions provides a sharp contrast to the conception of mammalian parturition held until recently. This conception was based on the known fact that oestrogenic treatment lowers the threshold of uterine stimulation to oxytocin and progesterone inhibits this effect. The essence of the theory was that towards "term" luteal secretion is reduced and the relative (or absolute) increase of oestrin raises the sensitivity of the uterine muscle until it is activated by the oxytocin always circulating in the blood. An increase in the level of oxytocin, as occasionally recorded by some workers, would form an additional safeguard to the safe working of this scheme. Doubt was first thrown on this scheme by Smith's (1932) observation that hypophysectomized rats delivered their litters successfully. Smith aimed at removing the posterior pituitary alone while leaving the anterior lobe intact. Fisher *et al.* (1938) have pointed out that if the anterior lobe is left *in situ* the forward extension of the posterior lobe must be left intact also, so that Smith's results do not provide unequivocal evidence for the non-participation of oxytocin in parturition. Fisher found that completely hypophysectomized cats did not deliver their litters normally and in some cases not at all. He concluded from this that the non-essential nature of oxytocin had not been established. Robson (1936) and others have shown that when pregnant rabbits are completely hypophysectomized they can deliver their litters. Selye *et al.* (1934) had found the same in rats. The conclusion seems clear that either there is an extra pituitary source of oxytocin or that pituitrin may be retained in the circulation for long periods. Clearly the data available do not justify far reaching comparisons, but at a time when doubt is being thrown on the role of pituitary oxytocin in the process of mammalian parturition

is doubly interesting to note that the pituitary is not essential for oviposition *Rana.*

While the above experiments do not rule out the participation of humoral encies in oviposition they do eliminate the more likely excitant substances.

) *Nervous control.*

Our next efforts were directed to a consideration of nervous control. Preinary experiments showed that electrical stimulation of either the general body rface of intact gravid animals or the upper end of the cord of decapitate preparations cited strong convulsions of the body wall and especially of the dorsal musculature a whole, sufficient in intensity to evacuate the uterus in the absence of any check the lower (cloacal) end. No eggs, however, were passed out through the cloaca, ough in one preparation in which incision was made in the ventral body wall was noted that the convulsive contractions induced brought about bursting the uterus wall and escape of its contents into the surrounding body cavity.

A test to determine whether the extrusion mechanism was under nervous conl was made as follows:

Twenty-four pairs of coupling frogs were used immediately they were brought o the laboratory. The spinal cord of the female of each of twenty-one pairs was stroyed by pithing, care being taken not to dislodge the male. The remaining ree pairs were kept as controls. Each pair was then segregated in a flat-bottomed containing water to a depth of half a centimetre. The three control females all truded their eggs within 3 days of segregation. Of the experimental frogs twelve ales were still coupling after 7 days and eight males continued to couple for 14 ys. None of the twelve females of these pairs extruded their eggs. With one ception none of the other pithed females extruded their eggs, though four of their mber survived for 3 weeks after pithing. This one exception was found to be completely pithed. While these results suggested nervous control, all attempts to cit extrusion by electrical stimulation by the procedure followed in the preinary tests gave the same negative result.

Study of relevant literature disclosed little beyond a suggestion of Wiedenbaum 894) that expulsion is brought about by contraction of muscle fibres of the uterine all. Microscopic examination of sections and whole mounts showed little evidence r this when the enormous mass of eggs carried in the distended membraneous c is borne in mind. Apart from its sac-like expansion into a "uterus" and the arcity of cilia in the lower oviduct in *Rana* previously referred to, there appeared be no significant difference in the musculature of the oviducal tube generally tween *Rana* and *Xenopus.*

Accordingly a re-examination was made of the whole pelvic region with the aid serial sections, dissections and experimentally. The results were as follows:

(1) In *Rana* as in *Xenopus* the ultimate outlet of the cloaca is controlled by a hincter band of smooth muscle.

(2) In *Rana* the urostyle is extended backwards so that its terminal portion tends over nearly the whole length of the cloacal tube in close proximity to the

dorsal surface of the latter. A section through this region (Fig. 1) just anterior to the level of the uterine apertures shows ureters and uterine exits lying closely beneath the urostyle between the latter and the rectal tube. Beneath the cloaca lies the pelvic symphysis. Very slight downward pressure from the urostyle, therefore, brings the cloacal tube into contact with the pelvis in the mid line, and immediately compresses the cloaca in the region of the openings of the three types of duct which enter it. The passageway betwixt urostyle dorsally and pelvic groove ventrally is at best a meagre one, and its dilation to allow of the expulsion of either a faecal

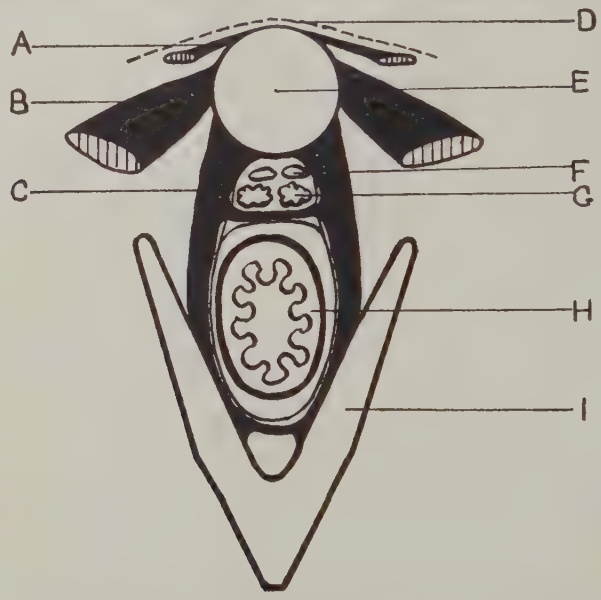


Fig. 1. Transverse section through cloaca of *Rana* slightly anterior to oviducal ("uterine") openings. The cloaca lies in the groove ("Beckenhöhle") formed by union of the ilia immediately below the hinder end of the urostyle. Muscles in black: *A* = coccygeo-iliac, *B* = pyriformis, *C* = compressor cloacae, *D* = dorsal surface of body, *E* = urostyle, *F* = ureter, *G* = oviduct, *H* = rectum, *I* = ilium.

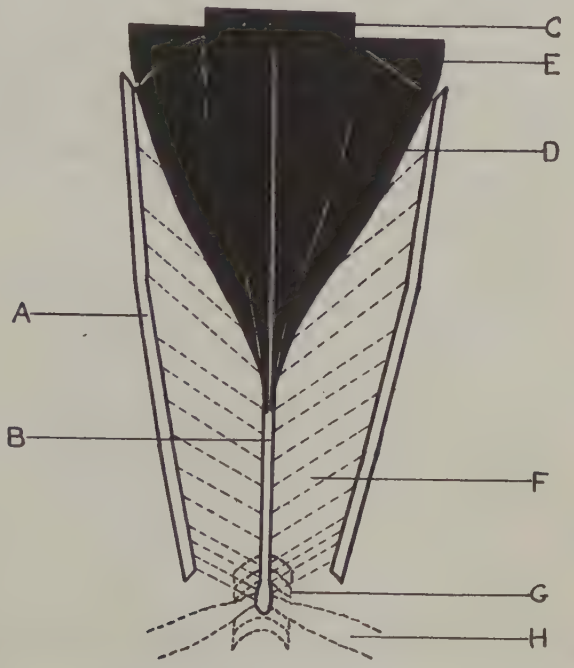
mass from the rectum or ova from the uterine apertures clearly implies a lifting of the urostyle and/or appropriate rotation in an anti-clockwise direction of the pelvis which in *Rana* articulates freely at the iliac sacral junction.

(3) Examination of the musculature (Fig. 1) showed that the cloaca is embraced by a symmetrically paired muscle, extending between the hinder part of the urostyle dorsally and the bases of the ilia ventrally. This muscle, the compressor cloacae (Ecker & Wiedersheim, 1904), supports the suspension of the cloaca from the urostyle. Bundles of fibres pass from the urostyle around the whole cloacal tube and a strong band passes around the bases of the uterine openings just above the entry of these into the cloaca and may be readily seen embedded in the thickened wall of the cloaca at this point, clear of and dorsal to the wall of the rectum.

(4) At the tip of the extended urostyle a pair of muscles extend outwards and are inserted along the femur on each side. These muscles are termed, by Ecker &

edersheim (1904), pyriform muscles. They obviously play a considerable role bracing the thighs firmly against the pelvic region prior to the act of leaping.

(5) Faradic stimulation applied at the tip of the urostyle of a pithed animal owed that the combined effect of the pyriform muscles and the compressor cloacae at of the former far exceeding the latter) was to pull the urostyle in a ventral rection hard down on the pelvis, clamping down the cloaca and completely cluding it. In this movement the coccygeo-iliac muscles (two thin sheets of uscle extending from urostyle to sides of the pelvis) also shared in a lesser degree.



g. 2. Principal muscles controlling urostyle in *Rana* from above. *A* = ilium, *B* = urostyle. Elevator uscles in black: *C* = longissimus dorsi, overlying *D* = coccygeo-sacral, which is reinforced by = ileo-lumbar, *F* = coccygeo-iliac, *G* = compressor cloacae, *H* = pyriformis.

A common position of the female frog during the act of oviposition is with the ighs extended backward, implying relaxation of the pyriform muscles. In this sition general stimulation in the intact animal and/or local stimulation of the lvic region of a pithed animal immediately resulted in complete occlusion of the oacal passage. The negative results of attempts to elicit extrusion by general ctrical stimulation are thus at once explained.

(6) A powerful opposition to the action of the foregoing muscles exists in the oup of muscles controlling the urostyle from the sacral region (Fig. 2). These are e longissimus dorsi and the coccygeo-sacral muscles, reinforced by the paired ileo-mbar muscles. Stimulation of these, following section of the pyriform muscles d the compressor cloacae, jerks the urostyle strongly upwards high above the pelvis.

(7) In animals in which the pyriform and compressor cloacae muscles have been sectioned slight stimulation of the ventral body musculature—in particular the pectoralis abdominalis—readily results in the hinder part of the pelvis being drawn ventralwise and slightly forward and, though in a smaller degree, also enlarges the gap between urostyle and pelvis.

It appears, therefore, that there exists a constellation of opposed skeletal muscles whereby the passage between urostyle and pelvis can be enlarged or *vice versa*, in that the action of the pyriform muscles and the compressor cloacae in occluding the passageway is balanced by the action of dorsal and ventral muscle groups in widening it in a dorso-ventral plane. It is clear from observation that the usual position of the female in oviposition at least facilitates the action of such a mechanism.

A series of experimental tests was then made on spinal *Rana* and repeated on spinal *Bufo* where the length and position of the urostyle are approximately the same as in *Rana*, but where pelvic movements are somewhat less free owing to the slightly dilated processes of the sacrum overlapping the ileo-sacral junctions. The results may be tabulated as follows:

Position of electrodes A and B		Response	
A	B	*Rana*	*Bufo*
1. Base of urostyle on spinal column	As A	Slight lift of urostyle	Similar
2. Front boundary of sacrum	As A	Maximal lift of urostyle, cloaca considerably stretched dorso-ventrally	Marked lift of urostyle only
3. Midway between sacrum and skull	As A	Lift of urostyle only	Maximal lift of urostyle, cloaca stretched dorso-ventrally
4. *Rana:* Front boundary of sacrum *Bufo:* Midway between sacrum and skull	Right axilla	Local response only of adjacent body wall	
5. Do.	Slightly posterior to axilla	Do., but slightly more extensive. No obvious movement of urostyle	
6. Do.	Posterior to axilla on dorso-lateral abdominal wall approx. at the level of vertebrae VI–VII	Maximal lift of urostyle combined with slight movement of pelvis ventralwise and marked convulsion of the whole abdominal wall	
7. Do.	Further back behind level of sacrum	Do., but less marked especially in regard to movements of abdominal wall	

These tests establish that appropriate stimulation will (*a*) dilate the urostyle-pelvis passageway to an extent far greater in degree than suffices for ordinary defaecation and simultaneously bring about spasmodic contraction of the abdominal musculature. The complexity of the ultimate distribution of the nerve fibres of the

al plexus is such that elucidation of the precise part played by separate muscles olved is difficult. Their combined action is, however, clear. When the urostyle ised to its maximum extent the action of part of the compressor cloacae muscle nerly suggested (Ecker & Wiedersheim, 1904) seems to be well founded, i.e. the dorsal wall of the cloaca is pulled down towards the external orifice, thus ging the region which receives the oviducal and urinary apertures closer to the rior and slightly shunting the anal opening of the rectum in a ventral and rior direction.

Repetition of the foregoing tests on intact female *Rana* resulted, when the rodes were applied as in experiment no. 6 in the above table, in the cloaca being ned widely and the immediate extrusion of paired masses of the characteristic -like material which is normally present in the lower oviducal tract of breeding ales. Dissection confirmed that the animals were approaching the gravid condi-, with enlarged oviducts which along with the uteri contained copious secretion.

Position of the male

In the light of these findings the possibility previously mentioned that pressure the male on the body of the female may aid in oviposition—since gentle ssure applied to gravid frogs occasionally results in extrusion—may be further sidered.

(1) In *Rana* the male's clasp is axillary. His body pressure is therefore on the al surface of the female from the sacrum forwards. His arms clasp the body of the female anterior to the level of the sacrum. In the normal coupling ition in water a tendency for the male to slip backwards slightly owing to gravity be observed.

The remarkable increase in sensitiveness to faradic stimulation of the pectoral on of the breeding male (Smith, 1938) appears very significant in the mainte-ce of a position which does not interfere mechanically with the lifting movement he female urostyle and associated pelvic movements necessary for extrusion of ine ova, and may be, indeed, of great significance in normal oviposition.

In *Xenopus* the male's position is posterior to the sacral region. In this position pectoral region rests on the female's urostyle and his arms clasp the anterior ic region. Compared with the condition in *Rana* the whole sacral region in *opus* is relatively immobile; urostyle, sacrum and anterior pelvis are firmly nd together and their rigidity is reinforced by large paired wing-like outgrowths he sacral region, which carry far back between the extended ilia the rigidity of sacral bridge (Fig. 3). The condition in *Xenopus* approaches that of *Pipa*. In the er the urostyle is fused with the sacrum as in *Breviceps*, *Phyllomedusa* and *bates* (Ridewood, 1897). In *Pipa* the transverse processes of the sacral region luding the first post-sacral vertebra (Ridewood)) are greatly dilated. In *Xenopus* urostyle is fused to the sacrum and the broad distal ends of the transverse pro-es of the latter meet the inner faces of the extended ilia, contributing greatly he firmness of the pelvis, e.g. when clasped by the male. The firmness of the

forward pelvic region is in marked contrast to the condition in *Rana* where iliac-sacral articulation is remarkably free and in *Bufo* where the sacral processes are somewhat dilated to cover the tips of the ilia, but do not extend backward between them.

The massiveness of the musculature associated with the urostyle and lumbar region in *Xenopus* is such as readily to support dorsal pressure by the male. Thus he exercises no pressure on the cloaca itself. The urostyle presents a striking contrast to that of *Rana* and *Bufo* in that it ends well forward of the body of the pelvis, and does not overlap the cloacal tube (Fig. 3). Beneath the gap thus left between urostyle and pelvic symphysis the body wall is reinforced ventrally by an ypsiloid cartilage.

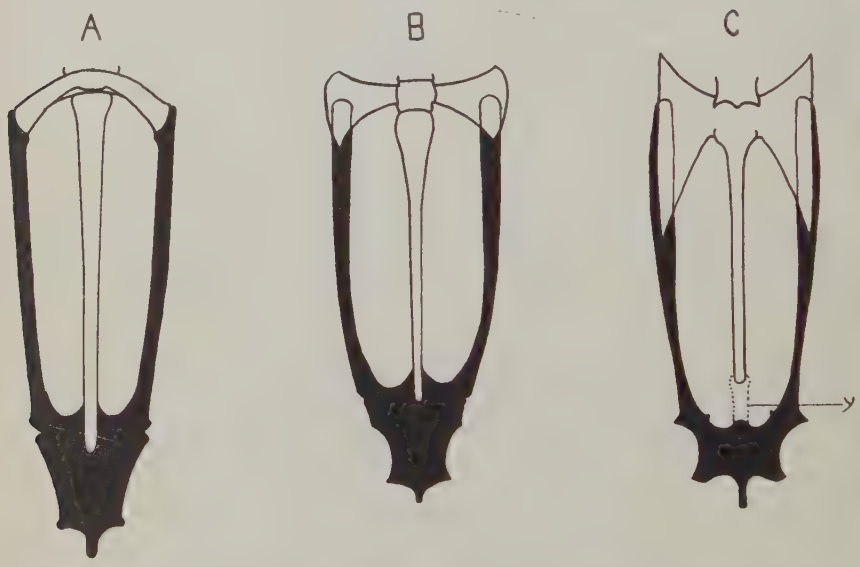


Fig. 3. A, *Rana*; B, *Bufo*; C, *Xenopus*; from above. *Y*, ypsiloid cartilage (ventral).

Consequently in *Xenopus* the cloacal tube lies on the pelvis and the products of rectum, oviducts and ureters pass freely through without interference from the urostyle. A thin suspensory band of fibres runs well forward under the skin from the dorsal lip of the cloaca to the urostyle tip, but we have not so far identified in *Xenopus* the very prominent pair of pyriform muscles which in *Rana* and *Bufo* run from the extended tip of the urostyle outwards to the femora.

GENERAL SUMMARY

1. The normal rhythm of sexual activity of both *Xenopus* and *Rana* is controlled by secretions of the anterior lobe pituitary. Progesterone and testosterone, as well as extracts of A.L.P., evoke ovulation in normal and hypophysectomized *Xenopus*. Injection of pregnancy urine into hypophysectomized *Xenopus* evokes enlargement of the reduced ovary and ovulation.

2. Serial injections of pituitary extracts and oestrin were without effect on the ount of egg jelly secreted.

3. Ovulation in *Xenopus* is immediately followed by oviposition. The eggs are ried by continuous ciliary tracts from the body cavity via the oviduct to the rior. In *Rana* ovulation is not immediately followed by oviposition. The eggs umulate in the lower third of the oviduct and are expelled *en masse*.

4. Experiments on extirpation of endocrines and the injection of appropriate racts, together with the responses to pithing and electrical stimulation, lead to the clusion that oviposition in *Rana* is probably under nervous not humoral control. e muscular and skeletal mechanism involved in the retention and extrusion of s in *Rana* is described.

Acknowledgement is made to the Medical Research Council for their support his investigation, and to Organon Ltd. for gifts of oestrogens.

REFERENCES

AMSON, D., ROBERTS, S. M. & WILSON, P. D. (1934). *Surg. Gynec. Obstet.* **58**, 595.
LERBY, C. W. (1933). *Biochem. J.* **27**, 2022.
— (1934). *Nature, Lond.*, **133**, 493.
W, F. A. E. (1939). *Brit. med. J.* **1**, 766.
FREMERY, P., KOBER, S., & TAUSK, M. (1931). *Acta brev. Neerl.* **1**, 146.
ER, A. & WIEDERSHEIM, R. (1904). *Anatomie des Frosches.* Braunschweig: Vieweg und Sohn.
OLD, H. L. (1939). *Sex and Internal Secretions*, p. 966. London.
HER, C., MAGOUN, H. W. & RANSOM, S. W. (1938). *Amer. J. Obstet. Gynaec.* **36**, 1.
AW, F. L. (1925). *J. exp. Zool.* **42**, 411.
— (1929). *Physiol. Zöol.* **2**, 59.
GBEN, L. (1923). *Quart. J. Exp. Physiol.* **13**, 86.
— (1930). *Proc. Roy. Soc. S.A.* **5**, 19.
NDGREBE, F. (1939). *J. exp. Biol.* **16**, 89.
, S. H. & NOBLE, R. L. (1939). *J. Endocrinology*, **1**, 15.
RSH (1938). *Proc. zool. Soc. Lond.* **107**, 603.
RSHALL, F. H. A., VERNEY, E. B. & VOGT, M. (1939). *J. Physiol.* **97**, 128.
XWELL, L. C. (1934). *Amer. J. Physiol.* **110**, 458.
EWOOD, W. G. (1897). *Anat. Anz.* **13**, 359.
SON, J. M. (1936). *J. Physiol.* **86**, 415.
TAND, J. (1934). *Toads and Toad Life.* London: Methuen.
GH, R. (1935). *Biol. Bull. Wood's Hole*, **68**, 74.
— (1935). *J. exp. Zool.* **71**, 149.
— (1938). *Proc. Soc. exp. Biol., N.Y.*, **37** (4), 717.
NDERS, F. J. & COLE, H. H. (1936). *Proc. Soc. Exp. Biol., N.Y.*, **33**, 505.
YE, H., COLLIP, J. B. & THOMSON, D. L. (1934). *Anat. Rec.* **58**, 139.
PIRO, H. A. (1936). *J. exp. Biol.* **13**, 48.
PIRO, H. A. & ZWARENSTEIN, H. (1937). *J. Physiol.* **89**, 38*P*.
TH, L. (1938). *J. exp. Biol.* **15**, 1.
TH, P. E. (1932). *Amer. J. Physiol.* **99**, 345.
FER & HASLHOFER (1935). *Arch. Gynäk.* **159**, 313.
MS, H. (1936). *J. Amer. med. Ass.* **106**, 1364.
NA, S. (1930). *Biol. Bull. Wood's Hole*, **58**, 322.
DENBAUM, G. (1894). Inaug. Diss. Dorpat.
LFE, O. M. (1929). *Anat. Rec.* **44**, 206.
DEK, B. (1930). *Klin. Wschr.* **9**, 245.

THE MELANIN CONTENT OF THE SKIN OF *RANA TEMPORARIA* UNDER NORMAL CONDITIONS AND AFTER PROLONGED LIGHT- AND DARK-ADAPTATION. A PHOTOMETRIC STUDY

By BEN DAWES, D.Sc.

Department of Zoology, King's College, University of London

(*Received 27 September* 1940)

(With Five Text-figures)

INTRODUCTION

Few zoologists can have failed to observe the differences of tone and colour which are evident in the skins of freshly collected English frogs. Such differences of tone may be entirely unrelated to the state of aggregation or dispersion of melanin granules in the melanophores, because frogs which have been kept for some time in a vivarium under identical conditions of light intensity and background can still be grouped as pale, fairly dark, and dark individuals with closely similar melanophoral indices. Nothing is known about the quantitative basis of such tone variations, and one aim of the present work is to evaluate the amounts of melanin normally present in the skin of the frog by the use of relatively simple methods of melanin extraction and estimation.

Most workers on the chromatic response to photic stimulation have confined their attention to the state of aggregation or dispersion of pigment granules in the chromatophores, i.e. to the phenomena of "contraction" and "expansion" of melanophores. The melanophore index devised by Hogben is the means now usually employed for measuring the magnitude of movement evident in granules conceived to exist already. According to the work of Hogben & Slome (1936), the black and white background responses of Amphibia are dependent upon localized retinal elements. A substance "B", which is secreted by the pars intermedia of the pituitary complex, brings about the dispersion of melanin granules under conditions of overhead illumination in light-absorbing surroundings, while another hormone "W", which is secreted by the pars tuberalis or by some organ functionally dependent upon it, brings about the aggregation of such granules under conditions of overhead illumination in light-scattering surroundings. Attempts to detect the "W" hormone have not been successful so far (Abramowitz, 1939), and the American school favour a unihormonal theory.

Recent researches have tended to show, however, that the chromatic response has wider implications than merely the movements of pre-existing melanin granules, and involves increase or decrease in the total amount of pigment present in the skin,

east, after prolonged exposure to light-absorbing or light-scattering surroundings. at little evidence happens to be available is based upon counts of melanophores bak, 1910–13; Kuntz, 1916; Murisier, 1920–1; Ordiorne, 1933, 1936, 1937; and ers). While the possibility exists of variability in the amount of melanin in ividual melanophores, such evidence cannot be regarded as entirely conclusive. e more satisfactory method of chemical extraction and estimation of melanin has n used in the simplest possible form by Viltner (1931), who experimented with axolotls on white and black backgrounds respectively, and by Sumner & udoroff (1937, 1938), who met with only limited success, so that they were pelled to discard the method in favour of one involving counts of melanophores. The swiftness with which the chromatic response is evoked in fishes militates inst the possibility of processes of synthesis and degradation of melanin, because tyrosinase-tyrosine reaction is a slow one. It has been shown recently, however gben & Landgrebe, 1940), that the full effect of the chromatic response in *terosteus* is evident only when time graphs are taken into consideration. The ial stages of the background response are rapid, but the final stages are pro-ged. This is compatible with the idea of pigment synthesis and destruction, ause it permits of the requisite period for such changes. The present work was cuted partly to find out whether or not such additive and subtractive pigmentary nges are involved in the chromatic response.

Another aim of the present work is to compare spectrophotometric character-cs of the melanin of an amphibian with that of hair. Recent work has shown that ll likelihood, melanic pigments of the hair in various mammals and of melanoma chemically very closely similar if not identical (Zwicky & Almasy, 1935; sele, 1937; Daniel, 1938; Arnow, 1938). It is of some importance to ascertain ether or not such fundamental resemblances in the nature of melanin extend to melanin of an amphibian.

MATERIAL AND METHODS

The methods which have been adopted by the writer are not entirely original, but y are free adaptations of such methods as have proved satisfactory for the raction and estimation of melanin from hair, differing in several particulars. It uld seem that much of the difficulty which was experienced by Sumner & udoroff (1937, 1938) was due to their use of whole fish for the analyses, which e somewhat cumbersome. Such difficulty has been eliminated in the present rk by the use of stripped skin. Melanin extractions are based upon well-known perties of melanin to resist solution in acid or neutral solvents but to dissolve ly readily in alkalies, properties which were determined for the melanin of the glish frog by a series of experiments which need not be specified. The proteins of skin were broken down by digestion with pepsin in acid solution and not by drolysis with strong acid, as is necessary when the melanin is associated with atin (Einsele, 1937; Daniel, 1938; Arnow, 1938). The writer had no opportunity using a spectrophotometer, and the estimation of extracted melanin was effected

by means of a Duboscq colorimeter used in conjunction with suitable spectrum and neutral density filters. The use of this instrument may involve many sources of possible error, but all details of procedure were standardized so that any errors inherent in the technique are as uniform as they can be made. The work, it must be emphasized, is a preliminary study, which will be refined and amplified when opportunity permits.

ANIMALS

The English frogs (*Rana temporaria*) used in general analyses of the normal melanin content of the skin were taken from a vivarium as soon as possible after capture. The animals which were employed for study of the background effects were kept in glass containers wrapped externally with white or black paper respectively. The light-reflecting or light-absorbing surroundings of the animals were subjected to identical conditions of incident daylight. Animals and containers were washed in running water daily. None of the animals took food, but it is presumed that the effects of inanition upon the melanin content of skin, if any, were the same for all. No ill effects of captivity were evident after several weeks, and this may be attributed partly to the fact that any animal of apparent poor condition was rejected at the outset.

SAMPLES OF SKIN USED FOR THE MELANIN DETERMINATIONS

Samples of skin for analysis were obtained as follows. The animal was induced to urinate by gentle squeezing of the abdomen, after which it was blotted of water, weighed, pithed and skinned. The skin was removed in dorsal and ventral regions, which were first of all marked out by a horizontal incision along the sides of the body and the limbs as far as knee and elbow. Such a line roughly separates the more heavily pigmented dorsal from the more lightly pigmented ventral surface. All skin samples were carefully cleared of shreds of connective tissue and blood vessels, blotted of superfluous fluid and weighed. Preliminary experiments showed that drying agents, alcohol or air, impede processes subsequently made use of for breaking down the skin, and dry weights were sacrificed to easier destruction of the skin. Skin weights, it must be mentioned, were far too variable to be of any real value in this work.

The general tone of the skin was noted and the animals roughly classified as "pale", "medium dark", or "dark". The melanophore index of dorsal skin was read consistently, as was that of the skin in the web of the foot. Small fragments of dorsal skin were removed, one from the same situation in each sample, cleared in xylol and mounted in Canada balsam. These were used initially in attempts to determine light transmissions of whole skin samples. Two microscopes were focused and were linked by a comparison eyepiece. The mounted skin was placed on the stage of one microscope, a neutral density filter on the stage of the other. Both objects were covered with a thin slip of matt glass. Neutral density filters were changed one for another until visual matching of the halves of the field was achieved when various filters were used in apposition to the eyepiece. It was thought by this

ns to find a quick method of ascertaining the approximate density of whole ; the results showed, however, that it is not possible to make observations to n a density of 0·2–0·3, and the observations were discontinued because they ed to be little better than ordinary visual judgements. The mounted fragments of came to serve a useful purpose, however, for comparing counts of melanophores the densities of NaOH solutions of melanin from the same skins.

Excluding preliminary experiments which were devised to fix the necessary Hards, analyses were made of the amounts of melanin in the dorsal and ventral of sixty-five frogs (thirty-eight males and twenty-seven females). Eleven animals were employed in experiments to determine whether or not injections drenaline and post-pituitary extract produce any post-mortem effects on the nic pigmentation of skin. Experiments on the effects of hypophysectomy and e effect of repeated injections of post-pituitary extract were ruined by death of nimals during a period of high summer temperature. In view of the results h have emerged from experiments on the background effects, it now seems mely likely that well-marked reduction in the amount of melanin would result long-standing hypophysectomy. This matter awaits further experiments, h will be carried out at some future date.

SAMPLES OF EGGS AND EMBRYOS USED FOR MELANIN DETERMINATIONS

opulating male and female *Rana temporaria* were segregated and the fertilized were collected and placed in well-oxygenated tap water. Samples intended for sis were withdrawn from specific batches of eggs at intervals. At the outset, ggs formed one sample, but this number was reduced to sixty or less in some nces. Altogether, nearly 2000 eggs formed the basis of twenty-three estima- of melanin. The main purpose of these experiments was to prepare curves of ensity/wave-length and to compare these with similar curves for melanin from skin; in short, to compare the melanin from these two sources spectrophoto- ically. Each sample of eggs was boiled for a few minutes in distilled water, superficially in a stream of air, weighed and stored temporarily under HCl. The samples were treated with the minimum of delay exactly as were les of skin (*vide infra*), except that the concentration of pigment in the final H solution was adjusted to meet colorimetric requirements. These solutions standardized so that 90 c.c. contained the melanin of sixty eggs.

EXTRACTION OF MELANIN

he weighed skin was placed in boiling distilled water and kept boiling for in. This treatment presumably destroys tyrosinase, and it washes out of the yellow and greenish pigments contained therein, but leaves melanin unaffected. skin, now white in colour except where melanophores darken it, was cooled and ted by means of pepsin. The enzyme used (commercial pepsin) was made up in HCl buffer at *p*H 2, and each dorsal or ventral skin received an ample quantity

(100 c.c.) of the 1% solution, to which toluol was added in standard amoun
During the period of incubation, the skins showed no signs of blackening, and it
presumed that post-mortem melanogenesis did not occur. Incubation proceeded
35° C. for 48 hr. After remaining overnight in the enzyme solution, the sk
commenced to disintegrate, slight agitation being sufficient to induce it to break u
into small fragments. After 24 hr. the skin was reduced to a fine cellular sedime
from which individual melanophores could be picked out with a pipette, and after
further 24 hr. the melanophores were disrupted, and all that remained of the sk
was a clear solution and a sediment of fine brown or black melanin granules.

Each digestion mixture was diluted subsequently with a standard volume
distilled water. After settling of the pigment, the supernatant liquid was decante
and set aside for a few minutes, while the remaining melanin was washed in distille
water by a process of decantation and centrifuging. The decanted fluid was the
centrifuged and the supernatant liquid was passed through a fine filter. In the ca
of dark skins, a very fine film of melanin particles collected on the filter; these we
washed and passed through into the final NaOH solution with the alkali. The fin
solutions were such that the melanin collected from a dorsal *or* a ventral skin w
contained in 50 c.c. 0·4% NaOH (Analar). The pigment granules did not all pa
into solution in the cold, but those which remained invariably dissolved short
after boiling commenced, showing that melanin from the frog is more readi
soluble in alkali than melanin extracted from hair. Daniel (1938) found gre
variability in the time of boiling needed to dissolve whole samples of melanin fro
the hairs of mice; this is not the case for melanin obtained from the skins of frog
Boiling was continued, however, for 15 min., after which the volume of the NaO
solution was brought back to 50 c.c. by additions of distilled water. The resulta
solution was never cloudy. Undoubtedly it contained impurities, but these did n
colour the solution and in no way affected colorimetric determinations. The colo
intensity of these final solutions varied greatly, as the ranges of density to b
mentioned presently will show; extracts of ventral skin were straw coloured, whi
those of dorsal skin varied between golden brown and deep brown. After sever
weeks in the dark, some melanin granules may emerge from such solutions, but th
can be returned to solution by slight boiling, or even warming. Apart from this, t
solutions remained perfectly clear and apparently unchanged for several month
Unlike the solutions obtained from fishes by Sumner & Doudoroff (1937, 193
they were eminently suitable for colorimetric observation.

CALIBRATION AND EXPERIMENTAL USE OF THE DUBOSCQ COLORIMETER

The Duboscq colorimeter was made easier to use by affixing a small mirr
obliquely beneath the vernier and its scale. This made it possible to read t
instrument from a sitting posture. Lighting and screening arrangements were
follows. A Phillips argenta (opal) lamp (60W., 210V., run at 210V.) was fix
$14\frac{3}{4}$ in. from the mirror of the instrument and an Ilford no. 810 light filter was set
the light path $12\frac{3}{4}$ in. from the light source. A screen of thick cardboard was bu

ound the lamp and a light tunnel of the same material was made to complete ening arrangements right up to the instrument, the front of which was enclosed screen of black paper. A further screen was placed around the eyepiece. lings were taken in a room which had black walls and lacked windows, and all ble precautions were taken to ensure accuracy of results. Special care was cised in allowing sufficient time for dark adaptation of the eye on entering the room and in avoiding visual fatigue, as well as other likely sources of error ned by Yoe (1928). Readings of the density of several test solutions by three pendent observers at the outset were in consistently close agreement.[1] All ngs of the vernier were made in the light of a small hand lamp, which completely screened during the time when the observations were being e.

Calibration of the Duboscq colorimeter was carried out in the following manner. *right* cup of the instrument was used for the colour standard, which was ared from Reeve's Indian ink (which contains pure C) in preference to a nin solution because the latter is said to fade with age (Sumner & Doudoroff, 1938). Tests showed this estimable fluid to be very stable to rather severe itions (dilution, heating, treatment with acid, etc.). The strength of the ard chosen after tests with melanin solutions prepared from the darkest and t skins obtainable was 0·029 % by volume ink. This was prepared by adding ps of the ink from a stalagmometer to 1 l. of distilled water, and its strength letermined by weighings of 100 drops of ink from the same stalagmometer and equal volume of distilled water. The standard showed no signs of deterioration many months.

The *left* cup of the colorimeter was filled to constant level with distilled water he effective depth adjusted to 1·00 cm. The depth of the colour standard in the cup was adjusted to produce visual matching of the halves of the field when a f Ilford neutral density filters were placed one at a time above the left cup and der. In this way readings of the depth of standard in the right cup correspond- o known neutral densities were obtained. Nine neutral density filters were to cover a suitable range of densities; their density values were 0·1, 0·2, 0·3, 0·9, 1·2, 1·5, 1·8 and 2·1. This range of densities proved to be adequate for all melanin solutions examined.

Matching of right and left halves of the field of the colorimeter was effected for us wave-lengths of light by the further use of eight Ilford spectrum filters 601–8), which were set above the eyepiece so as to take in both halves of the Three readings of the depth of the colour standard required to match a par- ir neutral density filter and the 1 cm. depth of distilled water were made for each rum filter used, and the mean value was calculated. The results, a set of readings pths of colour standard corresponding to the specified neutral densities for each rum filter, were plotted on squared paper to a large scale (30 × 22 in.). They ded eight smooth curves, which were employed for transposing colorimetric

[1] The writer is indebted to Mr D. A. Kempson and Mr P. B. Bradley for their assistance in ng out this test of visual acuity.

readings subsequently made with melanin solutions into neutral densities, due rega being paid to particular spectrum filters employed at any time.

In the melanin estimations the only difference from the calibration experimen was the substitution in the *left* cup of the colorimeter of the NaOH solution melanin in constant amount set at the constant depth of 1·00 cm. for the distill water and neutral density filter. In these estimations, the mean of three readings f each spectrum filter was used except where individual readings varied by more tha 0·05 cm. (which was rarely), in which case three more readings were made and t mean of six calculated. The technique employed minimized movements of t colorimeter cups and the range for the right cup throughout the whole of the wo was relatively slight, 1·72 cm. down to 0·07 cm.

THE TERM "DENSITY"

The term "density" is used in this work according to the conception of Hurt & Driffield (1890) as log opacity or $-\log$ transparency. The relationship betwee the transmission of light and the thickness of the absorbing material (Lambert law) may be expressed (in logarithms to the base 10) as follows (Daniels, 1936):

$$E = \frac{1}{l} \log \frac{I_0}{I},$$

where l = the thickness of the absorbing layer (cm.), I_0 = the intensity of incide light, I = the intensity of transmitted light, E = the extinction coefficient (her density). A similar formula applies to the concentration c of the absorbing materi (Beer's law), viz.

$$k = \frac{2{\cdot}303}{c} \log \frac{I_0}{I} \quad \text{(Daniels, 1936).}$$

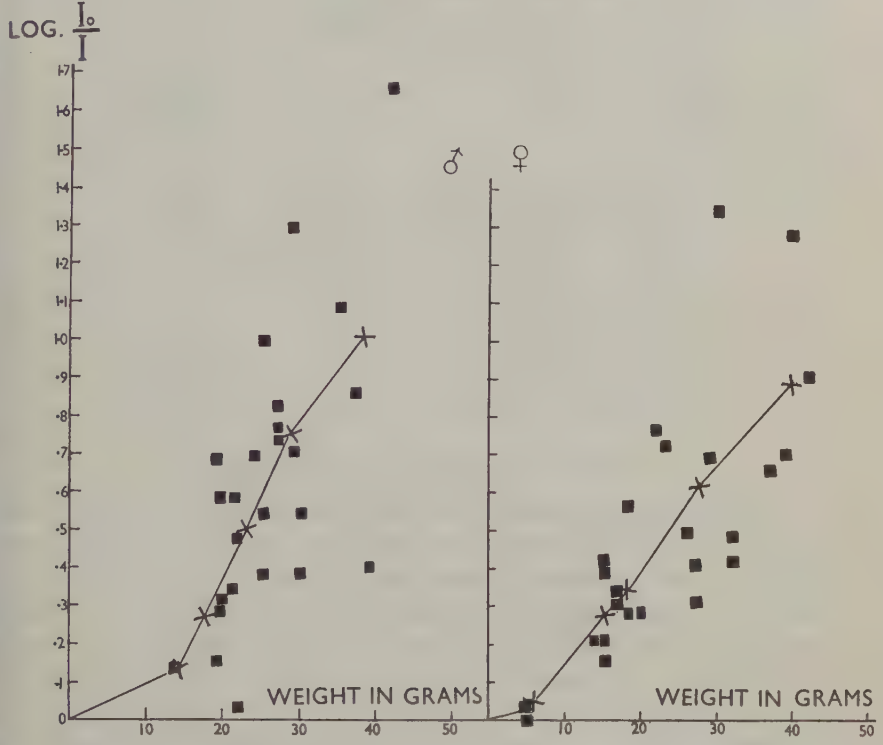
This applies to gases and solutions when dilution does not involve complicati change. It applies to solutions only when the solvent is transparent. In the writer estimations, the thickness of the melanin solution was kept constant at uni (1·00 cm.), the quantity of melanin solution per skin sample was kept constant 50 c.c., and the density was thus directly proportional to the concentration of t melanin derived from a single skin sample. In statements subsequently ma concerning the concentrations of melanin, mean density values for the eig spectrum filters are quoted. These must be more accurate figures than could obtained by the use of any one filter. In graphs, density values and their logarithi are plotted for wave-lengths at which the spectrum filters give maximum tran mission, namely, 4290, 4710, 4940, 5200, 5460, 5830, 6160 and 6870 A. The fir nought is omitted in readings shown on the wave-length scale of the graphs.

RESULTS

I. *The melanin content of the skin in relation to size*

If melanin is a product of metabolism which is stored in situations where it formed, the skin must contain more of this pigment in large frogs than in small on The relation between the melanin content of the skin and the size of the body is

arly shown by the available data because of wide variability in the small number estimations which could be made. Some of the twenty-four males and twenty-ur females which provided the data bearing on the melanin content of dorsal and ntral skin were selected for extreme duskiness or pallor so as to provide a measure the extent of variability. This partly explains the wide scattering of the points own in Fig. 1, where densities have been plotted against body weights. The



. 1. Plots of density/body weight (g.) for melanin solutions prepared from whole skins of male and ale *Rana temporaria*. The curves connect points representing the means of arbitrary size classes Table 1).

mplete range of densities evaluated is 0·13–1·29 (males) and 0·15–1·34 (females). nsities for the dorsal skin of an exceptionally pale and another exceptionally dark le (nos. 20 and 46 respectively) were 0·02 and 1·66 respectively. The density for dorsal skin of the darkest female was 1·34, while in one other female there was no preciable amount of melanin in the dorsal skin. Thus, it is plain that some dorsal ns contain ten times as much melanin as others and that in exceptional instances duskiness and pallor such a difference may be fiftyfold.

Despite such variability as this, it can be shown that melanin formation follows usual laws of growth. The curves drawn through the points shown in Fig. 1 nect mean values of density for arbitrary size classes (Table 1); in either sex, the ve is a portion of a sigmoid curve covering a fourfold increase in body weight. reme variation in the amounts of melanin in dorsal skin is probably unconnected

with normal growth. But it seems very probable that melanin in the dorsal skin increases relatively more rapidly than body weight. The dorsal skin of the mean male frog of 40 g. weight may contain more than three times as much melanin as the dorsal skin of the mean male of half this weight. Females considered over this size range show less than a threefold difference but more than a twofold one.

Table 1. *Data showing the relative melanin content of dorsal skin in relation to body weight*

No. of animals	Range of weights g.	Mean weight g.	Mean density of melanin solution per dorsal skin
♂ 1	10–15	14·0	0·13
4	16–20	19·5	0·29
8	21–25	23·0	0·50
7	26–30	28·5	0·75
4	30–	38·0	1·00
♀ 1	5–10	5·0	0·03
5	10–15	15·0	0·27
5	16–20	18·0	0·33
9	21–32	27·5	0·61
4	33–42	39·9	0·89

II. *The pigmentation of the ventral skin*

The ventral skin of the English frog is usually very pale, but it may be almost indistinguishable in tone and colour from the dorsal skin. The causes of such variability are unknown. As would be expected after such considerations have been made, melanin solutions prepared from ventral skin show a wide range of densities. In three males (53, 33 and 54) the ratio density of extract of ventral skin/density of extract of dorsal skin was evaluated as 0·04, 0·51 and 0·93 respectively; in three females (74, 26 and 28) the corresponding ratios were 0·09, 0·50 and 0·90 respectively. It is true to say, therefore, that ventral pigmentation ranges from equality with dorsal skin down to an almost pigmentless condition. Indeed, in five males and in three females, no melanin could be detected in the ventral skin. In one male, the dorsal skin contained less melanin than the ventral skin. Extreme pallor was the cause of this anomaly, the density for the dorsal skin being as low as 0·13 (no. 59; weight, 14 g.). In most individuals there is more than four times as much melanin in dorsal as in ventral skin; in thirteen males and fourteen females out of twenty-four individuals of either sex this was the case. Where pigmentation of the ventral skin is well marked, this skin may contain more than half as much melanin as the dorsal skin. The mean melanin content of ventral skin in either sex is 27% of the amount in dorsal skin (Table 2, lines 1 and 2). For equal weights of skin,[1] in either sex, the percentage is 44.

[1] The "ventral" skin invariably exceeded half the weight of the "dorsal" skin, despite the fact that the latter included the pigmented lateral as well as the truly dorsal region. It is likely that the thickness of ventral skin is much more than half that of the dorsal skin, though Francis (1934) provided the figures 0·2 mm. and 0·4 mm. respectively.

Table 2. *Data summarized for the various categories of individuals mentioned in the text, showing the relative amounts of melanin per skin, per unit weight of skin, and per unit weight of animal.* (*Mean values*)

Animals	Sex	No. of animals	Body weight g.	Weight of skin (g.)		Density of melanin solution per skin		Ratio $\frac{\text{Density}}{\text{Weight of skin}}$		Density per unit weight of animal (Dorsal skin)
				Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	
Mean	♂♀	24	26 ± 1	1·54	0·92	0·63 ± 0·08	0·17 ± 0·02	0·41	0·18	0·024
Mean	♂♀	24	26 ± 2	1·40	0·87	0·55 ± 0·06	0·15 ± 0·01	0·39	0·17	0·021
Spawning	♀	10	30 ± 2	1·79	1·04	0·64 ± 0·10	0·21	0·36	0·20	0·021
Non-spawning	♀	14	24 ± 3	1·25	0·84	0·48 ± 0·08	0·11	0·38	0·13	0·020
Dark	♂	6	33	2·26	1·26	1·12	0·18	0·49	0·14	0·034
Medium dark	♂	9	25	1·35	0·83	0·63	0·20	0·47	0·24	0·025
Pale	♂	9	23	1·25	0·78	0·30	0·13	0·24	0·16	0·013
Dark	♀	3	37	2·59	1·42	1·18	0·19	0·46	0·13	0·032
Medium dark	♀	11	27	1·59	0·98	0·57	0·16	0·36	0·16	0·021
Pale	♀	10	16	0·83	0·59	0·24	0·08	0·29	0·14	0·013
Black-adapted	♂ & ♀	8	23	1·10	0·68	0·67 ± 0·03	0·15 ± 0·02	0·61	0·22	0·029
White-adapted	♂ & ♀	9	19	1·05	0·84	0·42 ± 0·02	0·11 ± 0·02	0·40	0·15	0·022

III. *The melanin content of the dorsal skin*

The males used for estimations of melanin normally present in the skin rang in weight from 14 to 43 g., the females from 14 to 42 g. (plus a very small fema 5 g. weight). While mean weights are identical unless standard errors are taken in account, mean densities of melanin solutions prepared from the dorsal skin differ the sexes, the male possessing about 15% more of this pigment than the fema

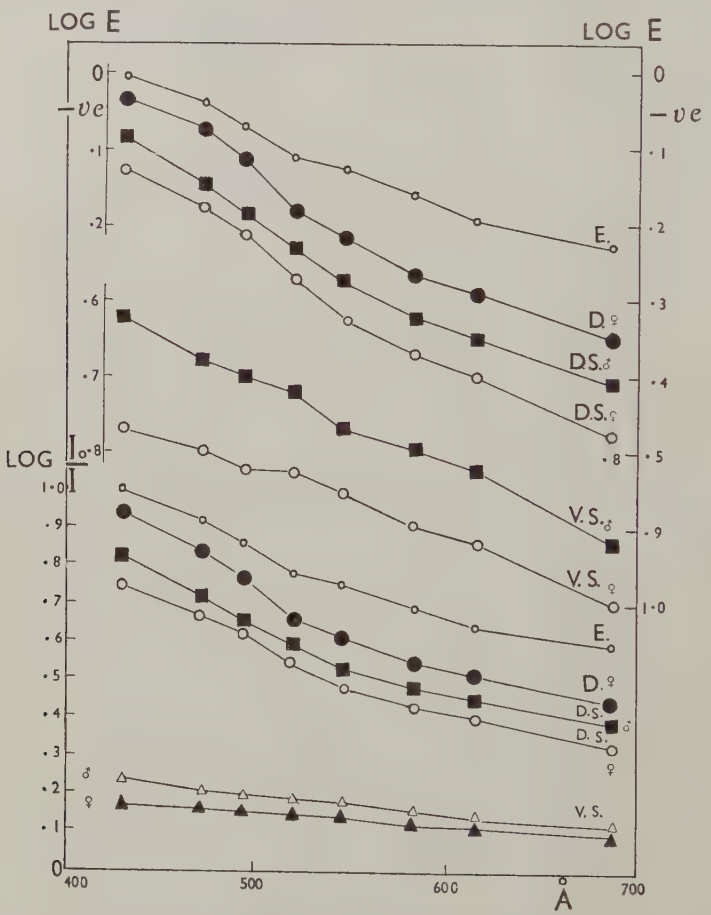


Fig. 2. Plots of density and log density against wave-length for various samples of melanin; *E*, fr the mean embryo; *D*, from the dorsal skin of the mean spawning female; *D.S.*, from dorsal sk *V.S.*, from ventral skin.

Consideration of the standard errors of the densities suggests, however, that t sexual difference in the degree of pigmentation may be more apparent than r (see Table 2, lines 1 and 2). The pertinent conclusion reached when the weight the area of the skin is taken into account is that there is no significant differen Fewer dark and more pale females than males were used for the estimations, a f which may account for the apparent difference.

Ten of the females were used immediately upon completion of the spawning .. They were mostly pale or only medium dark in the tone of the skin, but were ged with red and in some instances very red. The densities of the NaOH solutions melanin from the dorsal skins of these individuals greatly exceeded those of the maining fourteen individuals, the ratio of the two sets of densities being 4/3. The ference may be due to the greater mean weight of the females which had spawned. significant difference is evident if we calculate the density per unit weight of mal or of skin (Table 2, lines 3 and 4). The important conclusion can be drawn, wever, that the pale tone of the skin evident in association with a red coloration in males during the breeding season is not due to the lack of melanin, because this ment (or one which shares its light-absorption characteristics) is present at such ne in full amount.

Plots of log density against wave-length (Fig. 2) yield curves for the various lanin samples of which the slopes are uniform. From this fact, we can infer that significant chemical difference exists in the nature of the melanin which occurs in dorsal skin of males and females (*D.S.*) and females at spawning time (*D.*). The pes of the curves for ventral skin (*V.S.*) are not as steep as those for dorsal skin, t they show the characteristic absorption curve of melanin, namely, gradually reasing transmission from the violet towards the red end of the spectrum.

IV. *The correlation between visual judgement and estimation of the amount of melanin in the dorsal skin*

Animals were classified in the first instance by visual judgement of the tone of the n as "dark", "medium dark", and "pale" individuals. The density determinaons of melanin solutions made, they were reclassified as "DARK", "MEDIUM RK", and "PALE". The density ranges marking out the latter groups were greater an 0·8, 0·8–0·4, and less than 0·4 respectively. Such differences of pigmentation largely due to growth, the mean dark animal greatly exceeding the mean pale one weight (Table 2, lines 5–10). This dual treatment showed that the tone of the in is a fairly reliable if not an infallible index of the amount of melanin contained the skin, as the data in Table 3 indicate. Of forty-eight individuals which were assified as nine "dark", twenty "medium dark", and nineteen "pale", ten were DARK", twenty "MEDIUM DARK" and eighteen "PALE". The correlation between sual judgements and photometric determinations is not as close as these final mbers suggest, because five individuals classified as "dark" and four classified as pale" were found to be "MEDIUM DARK". In only one instance out of forty-eight, wever, was visual judgement erroneous by more than one-third of the total nount of melanin present in the dark skin. It is thus possible to judge by superial examination of the dorsal skin of the amount of melanin contained in it to thin one-third of its amount. This holds good whether the individual has been pt on a light-scattering or a light-absorbing background, or on one which has ither of these attributes, in which case the melanophore index provides no reliable easure of pigmentary differences.

Table 3. *Data showing the correlation between visual judgement and estimation of the amount of melanin in dorsal skin*

Re-classification according to density determinations

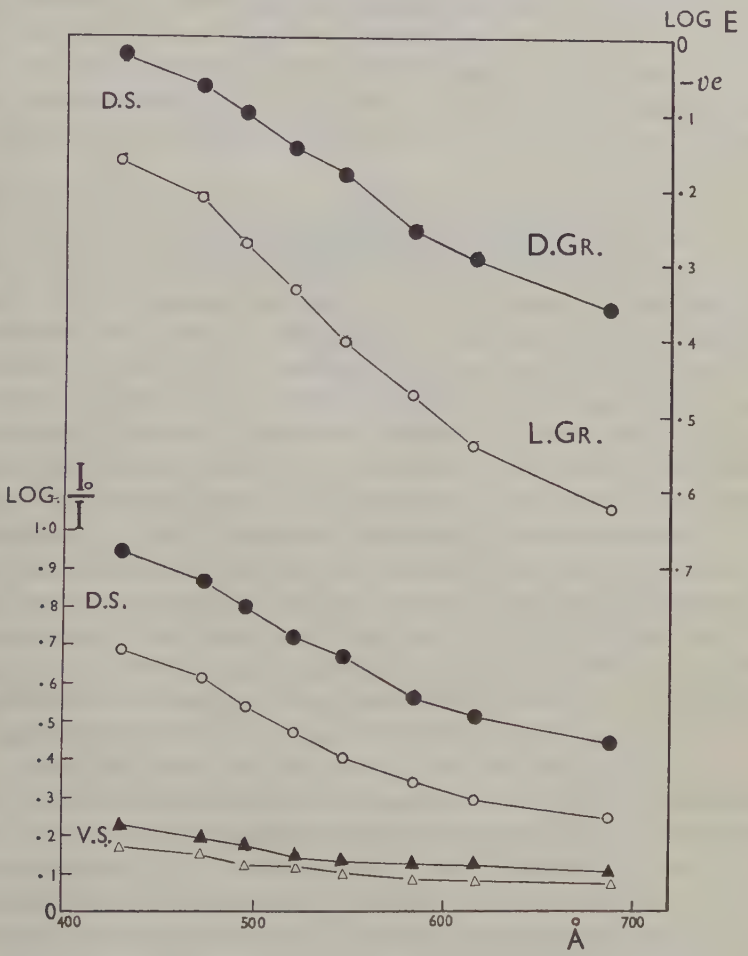
DARK, MEDIUM DARK or PALE	Mean range of density	Sex	No. of animals classed by visual judgement as			Total no. of animals
			Dark	Medium dark	Pale	
D.	–0·4	♂	2	4	0	6
M.D.	0·4–0·8	♂	4	3	2	9
P.	0·8–	♂	1	3	5	9
D.	–0·4	♀	2	1	0	3
M.D.	0·4–0·8	♀	1	8	2	11
P.	0·8–	♀	0	1	9	10
D.	–0·4	♂ & ♀	4	5	0	(9)
M.D.	0·4–0·8	♂ & ♀	5	11	4	(20)
P.	0·8–	♂ & ♀	1	4	14	(19)
Total no. of animals ...		♂ & ♀	(10)	(20)	(18)	

The curves relating log density and wave-length for melanin solutions obtained from "DARK", "MEDIUM DARK", and "PALE" individuals differ in their positions on the grid of co-ordinates and in their slope. The difference in log density between 4290 and 6870 A. ranges from 0·35 for "DARK" to 0·45 for "PALE" animals, i.e. the slope is slightly steeper in the latter. This slight departure from the parallel is not sufficient to signify any essential difference in the nature of the melanin present in the dorsal skins of such individuals. The results of such plotting indicate strongly that the essential difference between "DARK" and "PALE" individuals is a purely quantitative and not a qualitative difference in melanic pigmentation.

V. *The effect of black and of white background upon the melanic pigmentation of the dorsal skin*

Seventeen individuals, not included in the general estimates (three female amongst them) were kept on equally illuminated white or black backgrounds for several weeks. Under these conditions, individuals in light-reflecting surroundings pale considerably, while those in light-absorbing surroundings darken. At the end of a period of 5–6 weeks the two sets of individuals show extremes of pallor and duskiness. The aim of these experiments was to ascertain whether or not additive and subtractive processes are at work during this period, i.e. whether or not light-absorbing and light-scattering surroundings produce significant differences in melanic pigmentation when maintained for some time. Eight individuals (one of them a female) were kept on a black background for a mean period of 36 days; nine individuals (two of them females) were kept on a white background for a mean period of 33 days.

The outstanding result of these experiments, which are summarized in Table 2 es 11 and 12) and Fig. 3, is the much greater density of the melanin solutions pared from the dorsal skins of the animals which were kept on the black back- und. The dorsal skin of such animals contains, on the average, nearly 60% more anin than the dorsal skin of animals which have been kept on a white background



. 3. Plots of density and log density against wave-length for melanin solutions prepared from the n of frogs subjected for a period of weeks to black (*D.GR.*) and white (*L.GR.*) backgrounds; *S.* refers to dorsal skin, *V.S.* to ventral skin.

a corresponding period. Some of the excess is doubtless due to the larger size of e mean darkened animal, but there is no ambiguity in the general result obtained. nit weight of dorsal skin contains 50% more melanin in the case of the darkened imals than in that of the paled ones. The large standard error is attributable to con- erable size differences in all the animals concerned, with consequent large dif- rences in the amount of pigment contained in their skins. Comparison of the two rves relating density and wave-length for the mean animals of these experiments

(Fig. 3) with those for the mean animal which was not kept on any particular background (Fig. 2) shows that the former fall respectively above and below the latter. The mean animal (male-female) provides a curve which lies closer to the curve for the white-adapted animal than to that for the black-adapted one. These results show fairly conclusively that the light-scattering or light-absorbing nature of the surroundings exerts a profound effect upon the melanin content of the dorsal skin, the former decreasing and the latter increasing the absolute amount of pigment present. The slight departure from the parallel which these curves for light- and dark-adapted animals show is insufficient to point to any qualitative modification of the pigment. The dual effect of the background is quantitative, additive or subtractive.

Further evidence concerning the effect of white or black backgrounds was sought in counts of melanophores. The situation chosen for the counting was a constant area of the skin of the web of the foot between the last two (post-axial) digits. The results of numerous counts show that in animals which have been kept in black surroundings for periods up to 5 weeks, the number of melanophores is at least 12% and possibly more than 15% greater than the number counted in an equal area of skin from animals which have been kept in white surroundings for corresponding periods. Reliance cannot be placed on the actual figures, because the melanophores of dark-adapted animals with dispersed pigment granules are ill-defined and in consequence more difficult to count. But the tendency to undercount "expanded" melanophores would make it necessary to raise the percentage figure, and the general result expressed is all the more likely to be significant. A further observation which is important in relation to light-adaptation is the occurrence in the skin of animals which have been kept for periods of weeks on a white background of "islands" of skin which are devoid of melanophores but contain loose aggregates of melanin granules. Such "islands", which are very evident in the web of the foot, do not prove that disintegration and destruction of melanin occurs during prolonged light-adaptation, but they strongly suggest that this is the case.

The excess of melanophores of dark-adapted animals over those of light-adapted ones is smaller than might be expected from consideration of the densities of the melanin solutions if, as some writers insist, the numbers of such pigment cells provide reliable criteria by which to evaluate the amount of pigment present in the skin. This apparent discrepancy induced the writer to measure the sizes of the compacted masses of pigment granules in many melanophores of the same and different individuals, under conditions of consistently close packing. The sizes of such granular masses vary considerably. Thus, in a pale individual which had been kept on a white background and consequently gave a low density reading for melanin extracted from the dorsal skin (0·18), the pigment masses were extremely uniform in size and measured approximately 0·019–0·021 mm. in diameter. Another pale individual (also a male) submitted to a similar experimental treatment but containing more melanin (density 0·41) possessed melanophoral pigment masses of extremely variable size; the smallest ones were spherical and measured 0·021 mm. in diameter, while the majority measured 0·025 mm. in diameter and many large

l masses, which were not evident in the previous animal, measured as much as × 0·037 mm. Were it necessary, these findings could be amplified. They lead e conclusion that counts of melanophores do not provide reliable information rning the amounts of pigment contained in the skin unless the sizes of the ent masses are also taken into account. This would present no difficulty in the of light-adapted animals with aggregated pigment granules, but it would be igh impossible in the case of dark-adapted animals with dispersed pigment les. From such counts as were made by the writer it appears certain that amounts of pigment in the skin are evident as relatively large aggregates of les in the melanophores, as well as in enhanced numbers of such cells.

VI. *Post-mortem effect of injections of post-pituitary extract and adrenaline on the melanin content of the skin*

he following rather crude experiments on a possible post-mortem effect of pituitary extract on the melanin content of the skin were carried out in lieu of xperiments with whole animals which failed as a result of death of the animals rned. Six frogs were pithed and weighed. The hind limbs were severed from ody by a transverse cut immediately anterior to the pubes and were then ated by a median vertical cut through the symphysis. The *right* limbs, which d as controls, each received an intramuscular injection of 1 c.c. normal saline; *ft* limbs were given an intramuscular injection of 1 c.c. post-pituitary extract is and Co.). The limbs were placed in separate Petri dishes, the atmospheres of h were kept saturated with moisture by means of pieces of damp blotting paper a distance from the limbs. The dishes and their contents were incubated at C. for 48 hr., at the end of which time each skin was stripped from its limb in one , superficially dried, weighed, and used for a melanin determination in the ner previously described.

he density values of melanin extracts of the skin from pituitary-treated and ated limbs (Table 4) can scarcely be regarded as showing a significant difference they are considered in conjunction with their standard errors. Two points or strong emphasis, however. These standard errors are relatively large because nimals differed markedly in size and because, as a consequence, very different nts of melanin were under consideration. Further, the density value for the ct of melanin of skin from the pituitary-treated limb was in every instance er than that of the control. These considerations made, it seems extremely able that the difference in density on the two sides of the body (27 % more nin per skin of the pituitary-injected limb, or, 33 % more melanin per unit ht of skin from the injected side) is significant, i.e. that intramuscular injection ost-pituitary extract enhances the melanin content of the skin under the itions specified.

he curves relating density and wave-length for the mean estimates of these riments (Fig. 4) are similar to those obtained in other instances but are some- steeper. This difference would be expected (if not to quite the extent found)

Table 4. *Data for experiments on the effect of injection into the freshly excised limb post-pituitary extract* (1–6) *and adrenaline* (1*a*–5*a*) *on the melanin content of ski The* left *limb is the treated one, the* right *limb is the control*

Animal no.	Weight of animal g.	Weight of skin (g.)		Density of melanin solution per skin		Ratio $\frac{\text{Density}}{\text{Weight of ski}}$	
		Left	Right	Left	Right	Left	Right
1	15·8	0·50	0·55	0·42	0·33	0·84	0·60
2	12·5	0·45	0·41	0·25	0·18	0·56	0·44
3	18·4	0·67	0·75	0·55	0·47	0·82	0·63
4	6·8	0·20	0·25	0·08	0·06	0·40	0·24
5	16·6	0·67	0·60	0·52	0·42	0·78	0·70
6	8·8	0·25	0·30	0·14	0·11	0·56	0·37
Mean	13·1	0·46	0·48	0·33 ± 0·08	0·26 ± 0·07	0·72	0·54
1*a*	17·5	0·48	0·33	0·84	0·74	1·75	2·24
2*a*	14·1	0·45	0·37	0·36	0·43	0·80	1·16
3*a*	14·0	0·42	0·22	0·52	0·49	1·24	2·23
4*a*	12·6	0·57	0·53	0·57	0·53	1·08	1·66
5*a*	13·0	0·50	0·25	0·65	0·62	1·30	2·48
Mean	14·2	0·48	0·30	0·59 ± 0·08	0·56 ± 0·05	1·23	1·95

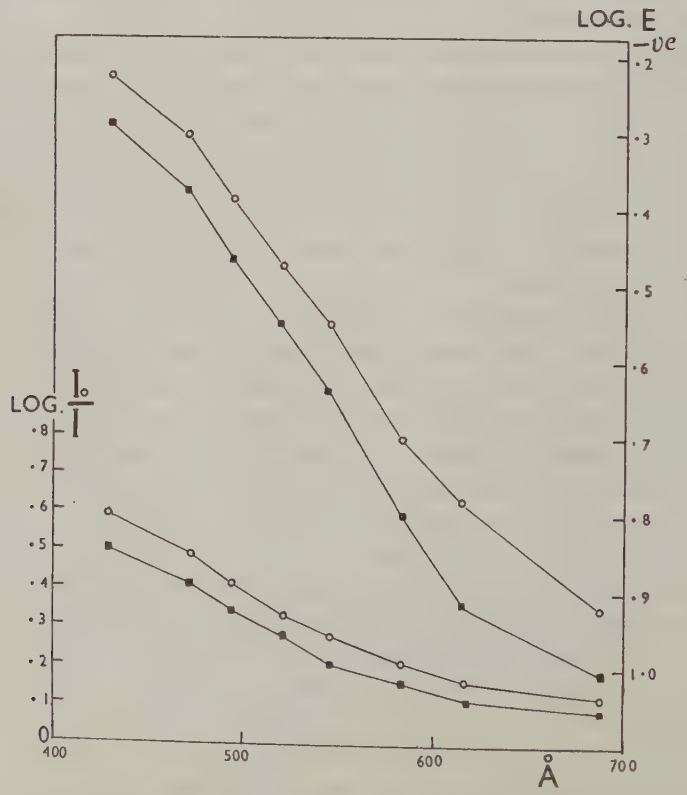


Fig. 4. Plots of density and log density against wave-length for melanin solutions prepared from skin of the leg 48 hr. after intramuscular injection into the excised limb of post-pituitary extr (circles). Control injected with normal saline (black squares).

m a consideration of the fact that pale individuals were chosen as the likeliest to ow any possible effect in these experiments, because, as has been shown, the slope the curve is steeper in pale than in dark individuals. The fact that the curve for the ntrol animal is of the same steepness shows that this slight departure from the neral results obtained cannot be due to the extract which was injected.

Five individuals were treated similarly to those given injections of post-pituitary tract, except that they received 1 c.c. 1/1000 adrenaline, injected intramuscularly to the *left* limb. After 24 hr., during which no visible difference in the tone of the in developed (the skin shows obvious darkening after injection of post-pituitary tract), the skins of the left limbs were treated with an additional 1 c.c. of the renaline solution and were incubated for a further 24 hr. The densities of the elanin solutions prepared from the skins of adrenaline-treated and untreated mbs (Table 4) show no significant difference when the standard errors are taken to account, though in four out of five instances the density corresponding to the left in is greater than that corresponding to the right skin. This result is puzzling, cause adrenaline produces pallor in the living animal. Another puzzling feature of ese experiments is the greater weights of the skins of the left (injected) side, a fference which is not attributable to inequality of size but which may be due to eater powers of water retention in the skin of the limb that received adrenaline. he negative conclusion reached after consideration of these results is that intra-uscular injection of adrenaline produces no effect on the melanin content of the in under the conditions specified. Certainly, there is no evidence of the degrada-on of melanin.

VII. *The melanin content of embryos of* Rana temporaria

Melanin estimations were made for embryos from four broods of eggs (A, B, C nd D), covering blastula, gastrula and neurula stages, and also an early stage during ne formation of external gills. The results (Table 5) show that the amount of

Table 5. *Data bearing on the relative melanin content of specified embryos of* Rana temporaria

Stage in development and sample	Number of analyses	Mean number of embryos per analysis	Mean density of melanin solution	Density per mg. embryo per c.c. NaOH solution
Blastula A	6	123	0·61	0·64
Blastula C	3	60	0·70	0·73
Gastrula A	5	113	0·77	0·54
Gastrula B	3	60	0·86	0·72
Neurula A	2	60	1·00	0·53
Neurula B	2	24	0·91	0·76
External gill D	2	55	0·91	0·83
Mean blastula	9	102	0·64	0·70
Mean gastrula	8	93	0·80	0·59
Mean neurula	6	46	0·94	0·63

melanin present in the egg during the earliest stages of development may vary by more than 15 % and that later stages show varying amounts of melanin, which may arise partly out of initial differences but may be due partly to varying rates of melanin production. Melanin formation, which must be considerable during gastrulation, seems to fall off relatively to weight increase in the gastrula, but to keep pace with weight increase or surpass it in the neurula and later stages. When mean densities are grouped so as to represent the three stages, blastula, gastrula and neurula, without regard to brood, it appears that the transition from blastula to gastrula involves a 25 % increase in the amount of melanin and the transition from gastrula to neurula a further increase of more than 17 %. The elongating neurula contains roughly half as much melanin again as the blastula (Table 5).

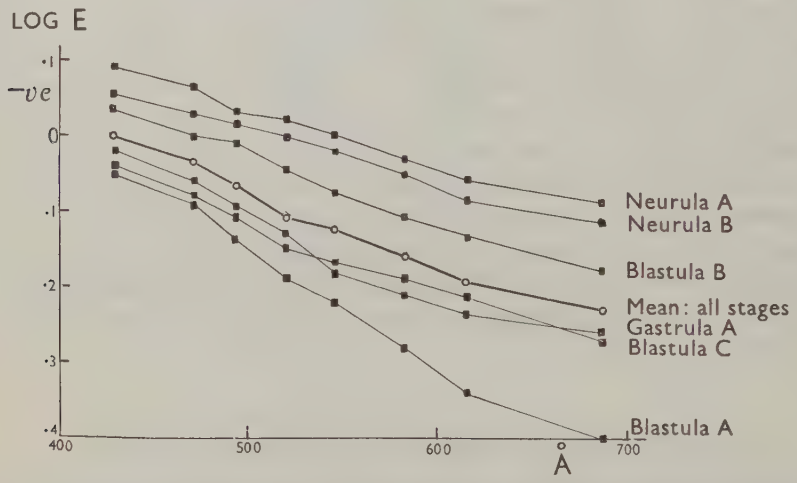


Fig. 5. Plots of density against wave-length for the various developmental stages of *Rana temporaria* specified.

There is a slight divergence from the parallel in the curves of log density/wave-length at the red end of the spectrum. The mean curve for all stages (Fig. 5; see also Fig. 2*E*) is less steep than the curve for melanin from the dorsal skin of the adult frog. The most substantial departure from the parallel is shown by the curve for blastula A, which is almost identical with (slightly steeper than) the curve for melanin from the dorsal skin of the mean male. These considerations lead to the conclusion that the pigment of these embryos is identical with that of adult skin.

DISCUSSION

In recent years, several workers have brought forward spectrophotometric evidence which proves the chemical identity of several apparently different melanotic pigments. Zwicky & Almasy (1935) prepared alkaline solutions of red, black and white pigments from coloured hairs of the horse, and of the pigment of horse melanoma, and concluded after comparison of light extinction data that all these

ments are spectroscopically indistinguishable, showing gradually increasing
t transmission towards the red end of the spectrum but decreasing transmission
he violet and ultra-violet. Arnow (1938) re-interpreted their data and disagreed
h their main conclusion, affirming that the distinctive colour of red human hair
lue to the presence of an oxidation product of melanin. Neumann (1937)
hed the conclusion that black, brown and yellow melanins from the hairs of
erent races of rabbit are chemically so closely similar that they must have a
mmon origin. Einsele (1937) extracted the melanin from hairs of mice of the
no series, and found that the pigments from different genotypes differ quanti-
vely in their light absorption as measured by a Duboscq colorimeter. Daniel
38) made a spectrophotometric study of such melanin samples and found that
ht out of eleven genotypes yielded qualitatively identical absorption spectra.
e concluded that colour differences in members of this albino series are not due to
erences in the chemical nature of the melanin, but must be due to variations in
quantity and distribution of the pigment. The absorption spectra of the three
aining melanin samples of her series were not so dissimilar as to account for
nounced colour differences.

It is both interesting and important to compare and contrast the melanin
orption curves prepared by Daniel (1938) for mice, by Zwicky & Almasy (1935)
the horse, and by the present writer for the frog. Those of the previous writers
w certain differences of slope within the limits of the visible spectrum, and one
o. 4; black hair: Zwicky & Almasy) is anomalous, signifying increased trans-
sion in the blue, slightly decreasing transmission in the green and yellow, and
ther slightly increasing transmission in the red (see their fig. 1). Otherwise, the
ves are of gradual slope, those of Daniel being straight lines, except in one
tance (**AABBcc**; see her fig. 1). Unfortunately, numerical data are not presented
d the curves reproduced are too small to permit of accurate comparison. As far
can be made out, the total differences in log density between the violet and red
ds of the spectrum are 0·7–0·8 (Daniel) and 0·5 (Zwicky & Almasy). The corre-
nding difference which has been demonstrated in the present work varies slightly
different samples of melanin; in melanin from the dorsal skins of normal animals
s about 0·35 while in melanin from the skins of light-adapted animals it is as
ch as 0·5. In pituitary-treated animals the difference is quite as considerable as in
ouse melanin. In general, therefore, the slope of the curve for frog melanin is less
ep than that of the curve for mouse or horse melanin, but in certain instances it
ains the same steepness. In the general comparison, horse melanin is intermediate
tween mouse melanin and frog melanin as regards the steepness of the smooth
sorption curve. Daniel reached the conclusion that melanin of the mouse is not
ential different from that of the horse. The present writer has shown that the
ference between frog melanin and horse melanin is less than that between mouse
lanin and horse melanin, when light absorption data form the basis of comparison.
e conclusion is reached that melanin of the frog is very closely related to (if not
solutely identical with) melanin of both mouse and horse. Equally close re-
mblance exists between the melanin of the frog and that formed from "dopa", as

well as the red-brown pigment extracted from red human hair by prolonged boiling with 0·1 *N* HCl (Arnow, 1938).

Turning now to consider the effects of light- and dark-adaptation on the melanin content of the skin, it must be noted that although an extensive literature exists there is a dearth of satisfactory quantitative studies. The subject has been under recent review (Sumner, 1940*b*). Most of the results in this field of investigation are expressed in terms of numbers of melanophores. As far as the writer is aware, no attempt has ever been made to evaluate the amount of melanin contained in such cells, so that counts of melanophores provide at best inconclusive data.

What has been stated already about the nature of the background response might be amplified slightly at this point. There is unanimity of opinion that dispersion of melanin granules in the melanophores (black background response) occurs as a result of the secretion by the pars intermedia of the "B" hormone (see Abramowitz, 1939). The response is conceived as a movement of pre-existing granules, a conception which must be extended in the light of evidence which shows clearly that increments of melanin are made when the background response is maintained. Pigment cells of *Amblystoma* tend to increase in number when kept continually "expanded" and, after a time, may be 50% in excess of pigment cells which are kept continuously "contracted" (Babak, 1910–13). Flounders possess 30% fewer melanophores when kept for some time on a white background than when kept for the same time on a dark one (Kuntz, 1916). Melanophores of the dorsal fin of young trout which have been kept for a period on black and white backgrounds respectively are in the ratio of 680/295, and show an even greater difference in the caudal fin (Murisier, 1920–1). Increase in number of the melanophores does not prove that the absolute amount of melanin is increased, although it is good circumstantial evidence that this is the case. Viltner (1931) estimated the melanin extracted from two axolotls which had been kept for 17 months on black and white backgrounds respectively. Using the method of Piettre (1911), which does not preclude the possibility of post-mortem melanogenesis, he found four times as much melanin in the dark-adapted as in the light-adapted animal. Analyses have been attempted also by Sumner & Doudoroff (1937) working with *Gillichthys mirabilis* and later (1938) with *Gambusia affinis*. The rather cumbersome technique which these writers adopted yielded a brown fluid showing some cloudiness, and it had to be discarded before really satisfactory results had been achieved in favour of the method of counting melanophores. By the addition of $MgCO_3$ to the brown fluid extracted from fishes which had been kept for about two months on white, black, and three shades of grey background, and by drying the resultant materials and grinding them with cedarwood oil, these writers obtained a paste which could be placed in special cells for the purpose of taking photometric readings. From such data as they obtained, what they termed "'qualitative' (i.e. only roughly quantitative results)", the conclusion was reached that the amount of melanin varies inversely as the log of the albedo of the background (i.e. the ratio of light impinging on it to that reflected from it). The conclusion was supported by further data based upon counts of melanophores (Sumner, 1940*a*).

The present work affords conclusive evidence for the first time that prolonged posure of one of the lower vertebrates to illuminated surroundings which absorb t strongly (black background) results in a marked increase in the melanin ent of the skin. After exposure of frogs to such backgrounds for 33–36 days the al skin of the mean animal of 23 g. weight contains roughly 35 % more melanin the dorsal skin of the untreated animal. The skin of such an animal contains % more melanin (50 % per unit weight of skin) than that of an animal which has n subjected for a similar period to an equally illuminated background which ters light (white background). The conclusion reached is that prolonged dark- ptation achieves more than the mere dispersal of pre-existing melanin granules melanophores, namely, the synthesis of fresh melanin.

The next question to be considered is that of the participation of the pituitary uplex in this synthesis. Several researchers have shown that the post pituitary etion increases the intensity of melanic pigmentation. The grafting of the adult ophysis into larval *Amblystoma tigrinum* at the stage of sex differentiation is owed by rapid melanosis due to increase in the number of melanophores and to the deposition of fresh granules of melanin. The animal suffers obliteration s normal tone pattern and becomes "sooty black" (Burns, 1934). The formation lack or brown pigment from the products of secretion of the hypophysis has n observed by Roussy & Mosinger (1935), who claim that such melanin is vely produced in human beings during old age. The present work provides ience which tends to show that the "B" hormone is concerned in the frog with production of fresh melanin, as well as with the dispersal of melanin granules. der specified post-mortem conditions, injection of a freshly excised living limb n post pituitary extract results during 48 hours in an increase of 27 % per skin % per unit weight of skin) in the amount of melanin.

There is diversity of opinion regarding the nature of the white background onse (see Abramowitz, 1939), which is generally visualized, however, as the regation of melanin granules in the melanophores by an agent or agents which still in question. This conception is inadequate, because the present writer and ers have produced evidence which proves that prolonged adaptation to illumi- ed light-scattering surroundings leads to the degeneration of melanophores and degradation of melanin. Almost one-third of the melanophores of *Fundulus* are during four weeks of treatment on a white background, nearly two-thirds during weeks; a similar if less pronounced effect is produced in *Ameiurus* (Ordiorne, 3, 1936, 1937). In the writer's opinion, this effect must be distinguished from a ardation of development of pigment in young *Macropodus opercularis* and *mbusia* sp. when such animals are kept on a white background (1937). The one e involves the degradation of melanin, the other merely its failure to develop. The truction of melanin has been observed by other workers, e.g. in *Lebistes* a few days er transference from a black to a white background by Sumner & Wells (1933). ese writers could see with low magnification numerous degenerating melano- ores in the skin, and in sections they noted the passage of melanin into the epidermis. e present writer has observed the degradation of melanin microscopically in

the skin, and has estimated its amount analytically in melanin solutions. It i difficult to see how a failure of the "B" hormone could result in the degradation o this pigment; presumably, even an inhibitory agent such as has been isolated b Daneel & Shaumann (1938) would fail to produce this effect. This would seem t provide an objection to the unihormonal theory of adaptation to background. Th fact upon which the objection could be based scarcely lends support to the concep tion of dual hormones propounded by Hogben and his co-workers, but if the "W" hormone exists (its existence has not been proved, nor yet disproved by the uni hormonal school) it may be the agent which disrupts as well as aggregates melani granules during prolonged light-adaptation. If not the "W" hormone, then som other powerful agent as yet unknown must be at work under such conditions. Th removal of melanin from the body has been observed in human patients wit Addison's disease by Jacobson & Klingk Jun. (1934). Several paths are taken, th tubules and Henle's loops of the kidney, the reticulo-endothelial cells of live spleen and lymph nodes and the colon mucosa, as well as the skin. The mean whereby melanin is removed from degenerating melanophores of fishes and amphibi subjected to prolonged white-background treatment are yet to be ascertained.

SUMMARY

1. Melanin has been extracted from dorsal and ventral components of the ski of English frogs by a simple method involving the leaching out of water-solubl pigments, the peptic digestion of washed skin, and the centrifugation of unaffecte pigment. The melanin was estimated in NaOH solution with the aid of an indian ink colour standard and by the use of a Duboscq colorimeter calibrated agains suitable neutral density filters at various wave-lengths.

2. Melanin varies greatly in amount in dorsal skins, some of which contain te times as much of the pigment as others. In extreme duskiness and pallor, th difference may be fiftyfold. In spite of such variability, melanin formation seem generally to follow the usual laws of growth.

3. Ventral pigmentation varies more than dorsal, ranging from an almost pig mentless condition to equality with dorsal pigmentation. The mean amount melanin in ventral skin is about 27% of that in dorsal skin.

4. Melanin occurs in the skin of females at spawning time in large amoun Thus, the pale, red colour of the skin is not due to lack of the pigment. Melani does not differ significantly in amount in the sexes.

5. Melanin can be determined by careful visual judgement of the tone of th skin to within one-third of the total amount present in dark skin.

6. The melanin content of dorsal skin is increased or decreased when frogs a kept for some weeks in equally illuminated light-absorbing (black) or ligh scattering (white) surroundings. The skin of the dark-adapted animal contai 60% more melanin than that of the light-adapted one after 5 weeks of treatment

7. The melanin content of the skin of a limb is increased under post-morte conditions following intramuscular injection of post-pituitary extract into the fresh

cised living limb. An increase of 27% was determined. No effect is produced adrenaline under these conditions.

8. Prolonged adaptation to light-absorbing or light-scattering surroundings oduces a more fundamental effect than the mere dispersion or aggregation of e-existing melanin granules in the melanophores, namely, the production of fresh elanin or the degradation of this pigment. The subtractive process, which is ident microscopically, is due to some agent at present unknown.

9. Frog melanin is shown by its absorption characteristics to be chemically very osely similar to, and possibly absolutely identical with, mammalian melanin and the gment of melanoma.

The writer wishes to express his thanks to Prof. C. M. Yonge for providing him th the facilities for carrying out this research in the Department of Zoology, niversity of Bristol, and to Mr D. A. Kempson for much technical assistance. Ir P. B. Bradley assisted the writer with the early stages of the research.

REFERENCES

BRAMOWITZ, A. A. (1939). *Amer. Nat.* **73**, 208.
RNOW, L. E. (1938). *Biochem. J.* **32**, 1281.
BAK, E. (1910). *Pflüg. Arch. ges. Physiol.* **131**, 87.
—— (1912). *Z. Physiol.* **25**, 1061.
—— (1913). *Pflüg. Arch. ges. Physiol.* **149**, 462.
URNS, R. K. Jun. (1934). *Anat. Rec.* **58**, 415.
ANEEL, R. & SHAUMANN, K. (1938). *Biol. Zbl.* **58**, 242.
ANIEL, J. (1938). *J. Genet.* **36**, 139.
ANIELS, F. (1936). Photochemistry. In *Biological Effects of Radiation*, **1**, 253. Ed. Duggar, B. M. McGraw-Hill.
INSELE, W. (1937). *J. Genet.* **34**, 1.
RANCIS, W. L. (1934). *J. exp. Biol.* **11**, 35.
OGBEN, L. & LANDGREBE, F. (1940). *Proc. roy. Soc.* B, **128**, 317.
OGBEN, L. & SLOME, D. (1936). *Proc. roy. Soc.* B, **120**, 158.
URTER, F. & DRIFFIELD, V. C. (1890). *Journ. Soc. Chem. Ind.* **9**, 455.
COBSON, V. C. & KLINGK, G. H. Jun. (1934). *Arch. Path.* **17**, 141.
UNTZ, A. (1916). *Bull. U.S. Bur. Fish.* **34**, 173.
URISIER, P. (1920–21). *Rev. suisse Zool.* **28**, 45, 149, 243.
EUMANN, P. (1937). *Biol. Zbl.* **57**, 522.
RDIORNE, J. M. (1933). *Proc. nat. Acad. Sci., Wash.*, **19**, 329.
—— (1936). *J. exp. Zool.* **74**, 7.
—— (1937). *J. exp. Zool.* **76**, 441.
IETTRE, M. (1911). *C.R. Acad. Sci., Paris*, **153**, 782.
OUSSY, G. & MOSINGER, M. (1935). *C.R. Soc. Biol., Paris*, **119**, 931.
UMNER, F. B. (1940*a*). *J. exp. Zool.* **83**, 327.
—— (1940*b*). *Biol. Rev.* **15**, 351.
UMNER, F. B. & DOUDOROFF, P. (1937). *Proc. nat. Acad. Sci., Wash.*, **23**, 211.
—— —— (1938). *Proc. nat. Acad. Sci., Wash.*, **24**, 463.
UMNER, F. B. & WELLS, N. A. (1933). *J. exp. Zool.* **64**, 377.
ILTNER, V. (1931). *C.R. Soc. Biol., Paris*, **108**, 774.
OE, J. H. (1928). *Photometric Chemical Analysis.* New York.
WICKY, H. & ALMASY, F. (1935). *Biochem. Z.* **281**, 103.

THE STRUCTURE AND CONDUCTION VELOCITY OF THE MEDULLATED NERVE FIBRES OF PRAWNS

By W. HOLMES, R. J. PUMPHREY[1] and J. Z. YOUNG

From the Department of Zoology and Comparative Anatomy, University Museum, Oxford

(*Received* 1 *November* 1940)

(With One Text-figure)

INTRODUCTION

The observation that the nervous system of prawns contains fibres surrounded by a sheath which blackens on treatment with osmium tetroxide was first made in studies of *Palaemon squilla* by Retzius (1888, 1890) and Friedländer (1889). The descriptions which they gave of these remarkable fibres seem to have attracted little attention since their time, and the only more recent investigation on the subject is that of Nageotte (1916).

THE STRUCTURE OF THE MYELIN SHEATH

In the nervous system of the prawn *Leander serratus* upon which the present investigation[2] was made, almost all fibres have a sheath which blackens after osmium tetroxide treatment.

The relations of the myelin sheath with other parts of the fibre are essentially as described by Retzius in his later paper (1890) and by Nageotte (1916). The osmiophil layer does not lie directly upon the axon as in vertebrates, for the two are separated by a thin nucleated sheath. Nageotte described the nuclei of this sheath as being the nuclei of the myelin sheath, and he compared them with the Schwann nuclei of vertebrates, which he considered to be similarly the nuclei of the myelin layer. However, until we have further information as to the derivation and behaviour during development of the inner sheath and its nuclei, this homology with the Schwann sheath elements of vertebrates remains entirely speculative. Further light can be cast on the question of the nature of the inner sheath by consideration of the nerves of other invertebrates. Recent studies of the nerve fibres of crabs and other decapod crustacea have shown that even in forms in which the nerves are usually considered to be non-myelinated there is a layer of orientated fatty molecules in a position round the axon corresponding with that of the myelin sheath of the prawn (Schmitt & Bear, 1939). In some fibres of crabs this layer is sufficiently thick or dense to be visible as a thin black line after osmium treatment (Young, 1936, fig. 15). Thus the fibres of prawns differ from those of other Decapods only in that the sheath layer containing orientated lipoids is thicker

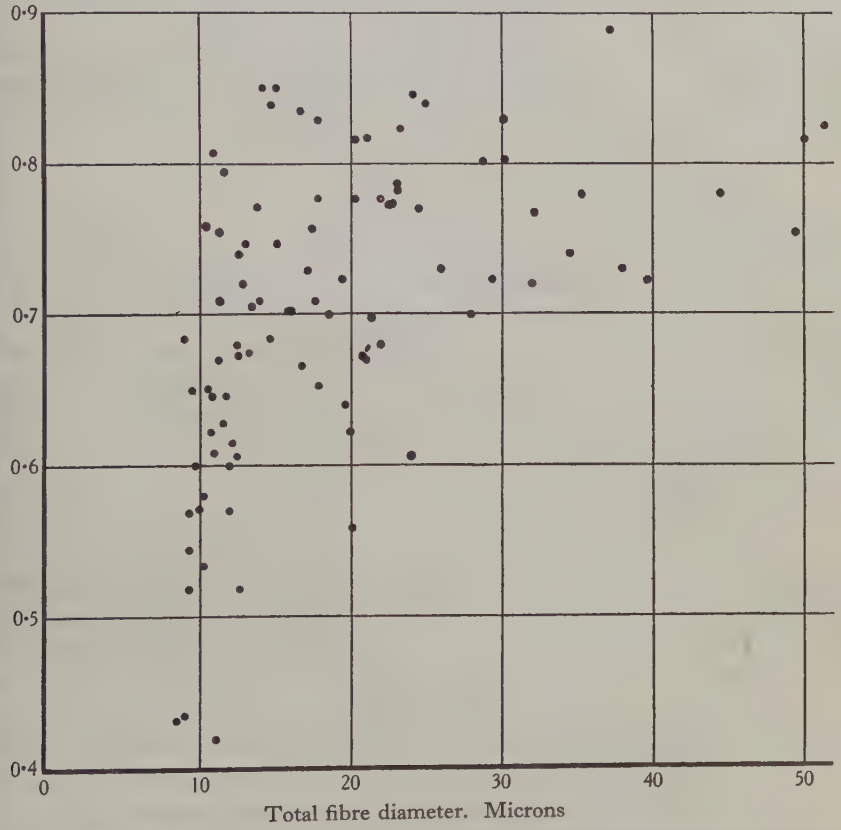
[1] Beit Memorial Research Fellow, Department of Zoology, Cambridge.

[2] The work was done at the Laboratory of the Marine Biological Association at Plymouth to whose Director, Dr S. Kemp, F.R.S., and staff, our thanks are due. Prof. E. S. Goodrich, F.R.S. kindly read the MS. of the paper.

probably contains a higher proportion of fatty to protein elements. Indeed, all s of crustacea and molluscs which have been carefully studied show this same gement of the sheaths, so different from that in vertebrates, and the prawn is liar among invertebrates only in the great development of the fatty layer.

SHEATH THICKNESS AND FIBRE DIAMETER

he problem of the relationship between the diameter of the nerve axon and the ness of its myelin sheath has been studied in vertebrates by Arnell (1936), itt & Bear (1936–7), and Gasser & Grundfest (1939); and in earthworms by or (1940). Both in vertebrates and in earthworms the thinner axons have



1. Plot to show the relative thickness of the myelin sheath in fibres of the central nervous stem of *Leander serratus*. The measurements given were made on transverse sections of two parate nerve cords.

tively thicker sheaths. The ratio axon diameter/total diameter (g) at first increases n fibre diameter and then becomes constant. For vertebrate fibres the plateau he curve is reached at total fibre diameter of from 6 to 8μ; for earthworm es at 40μ.

The accompanying graph (Fig. 1) shows the result of a series of measurements le on the fibres in the prawn central nervous system. Enlarged microphotographs

were made of transverse sections of the ventral nerve cord fixed in 0·5 % osmiu tetroxide solutions, and the measurements were carried out by means of a travellin micrometer microscope stage which carried a glass slide marked with fixed poin over the surface of the photograph. This method of course suffers from sever disabilities, particularly that fixation and staining may alter the thickness of th myelin sheath from that existing in the living fibre. The measurements show considerable variability of the axon/sheath ratio for fibres of the same size, yet th general relations resemble those in earthworms and vertebrates. The myelin relatively thicker on the smaller fibres. The ratio averages at 0·53 for fibres of 10 and less; at 0·69 for fibres between 10 and 20μ diameter. For the large fibre grou of total diameter from 20 to 50μ the trend is still further marked, and the rat averages at 0·77. This shows a smaller relative thickness of the myelin than is foun in any vertebrate fibres, corresponding with the unusually large total diameters the fibres. In the largest earthworm fibres, of diameter greater than 40μ, Tayl found a ratio as high as 0·9.

THE NODES

Both Retzius and Nageotte, when describing the prawn fibres, drew attentio to the fact that the myelin sheath is interrupted at intervals by structures closel resembling the nodes of Ranvier in vertebrate nerves. We find that in the nerv we have examined only the larger myelinated fibres have nodes, for we found no in fibres of a total diameter of less than about 13μ. The median giant fibres of th central nervous system, although they are connected with several cell bodies an are thus unlike simple neurons (Holmes, unpublished), also have segmental co strictions which may function as nodes although they are structurally unlike thos on the smaller fibres.

Retzius (1890) stated that at the node the myelin sheath is completely inte rupted, but Nageotte (1916) believed that the myelin, although much thinner at th node, was continuous over it. Recent investigations of the ultrastructure of nerv (Schmitt & Bear, 1939) have shown that no clear line of distinction can be draw between medullated and non-medullated fibres. For a myelin layer may be prese round a nerve although not sufficient in thickness or in density of fatty elements t blacken visibly on osmium treatment. Thus only the search for such a metatropi sheath at the node could determine whether the myelin is actually absent or merel thinned. We can, however, state that the myelin layer decreases in thickness at th node so much that it is no longer made visible by osmium treatment. The proba bility that it is actually completely interrupted is increased by the fact that in som preparations a distinct edge to the myelin sheath can be seen running transverse the axon on either side of the thinnest region of the node.

We have not found in the prawn any such correlation between internodal distan and fibre size as was described by Kubo & Yuge (1938) in vertebrates. The inte nodes vary very greatly in length even along a single fibre. In one case, for instanc four successive internodes along a fibre were 0·13, 0·22, 0·29 and 0·60 mm. length. Although in this case the internodes are shorter than those usually found

tebrates, we have also found in prawns nodes spaced as far apart as 3 mm., hout any corresponding change in fibre diameter.

THE CONDUCTION VELOCITY OF THE MEDIAN GIANT FIBRES

For a preliminary study of the conduction velocity of these invertebrate elinated fibres the median giant fibres of the central nervous system were used hnson, 1924). These fibres are convenient for experimental purposes as they pass hout synaptic interruption along the ventral nerve cord from the brain to the son, but they are unlike other fibres in the prawn in that they possess no Ranvier des. However, as has already been suggested, it may be that the segmental nstrictions of the axon in each ganglion have a function similar to that of more ical nodes. The diameter of the fibres studied varies considerably along the gth of the nerve cord, fluctuating between a maximum total diameter of about μ and a minimal diameter of 25μ. The average total diameter of the fibres used these determinations may be taken as 35μ, with a myelin sheath thickness of $4{\cdot}5\mu$.

To determine the velocity of impulse conduction in these fibres the whole ntral nervous system of the prawn was dissected out in sea water, and then lifted m this on the platinized platinum wire electrodes into a layer of paraffin oil. The mulating and recording system was essentially similar to that used by Pumphrey *al.* (1940). The stimulating electrodes were placed on one of the circumoesoageal connectives, and the recording electrodes on the posterior abdominal region the cord. A conduction distance of about 30 mm. was thus obtained. Conduction locities were obtained by measuring on the face of the cathode ray tube the stance between the stimulus artefact and the base of the spike for which the giant re was responsible. No difficulty was experienced in distinguishing this spike, it was quite separate from those produced by impulses travelling along the other ore slowly conducting pathways.

From determinations made in this way we found the median giant fibre to have conduction velocity varying between 18·4 and 23·0 m. per sec. at temperatures se to 17° C.

DISCUSSION

The method used for determining the conduction velocity suffers from the errors herent in any derivation of conduction velocity from measurement of the shock-ike interval; and the fact that the measurements were made in oil means that the corded conduction velocity is probably somewhat less than the normal value Hodgkin, 1939). However, neither factor is large enough to affect the conclusion at the nerve fibres of prawns conduct very much faster than do those of any rustacean yet examined. A crab fibre of diameter 30μ conducts at a maximum elocity of 5·5 m. per sec. (Hodgkin, 1939), and the greatest conduction velocity itherto recorded in a crustacean is one of 9 m. per sec. in *Homarus* (Monnier & ubuisson, 1931). We have found that in *Munida* a fibre of 50μ diameter conducts 6·4 m. per sec., and one of 56μ at 6·9 m. per sec.; and the fibres of this animal semble those of *Maia* and *Carcinus* in having a fatty sheath which just blackens

with osmium tetroxide. The fact that such a high conduction velocity is found in the prawns, whose fibres differ from those of other crustacea only in the greater development of the fatty layer gives great weight to the view that the value of a myelin sheath lies in the increased speed of impulse propagation which it makes possible. The only other invertebrate fibres which conduct at comparable rates are those of the earthworm, which are heavily myelinated (Eccles *et al.* 1932; and see Taylor, 1940), and those of the squid which are of very much greater diameter (Pumphrey & Young, 1938).

The largest prawn fibres are somewhat smaller than the largest fibres of the earthworm; both have sheaths of the same order of thickness, and their conduction velocities are similar. However, further details are needed before an exact comparison can be made. Although the prawn fibres conduct faster than those of other crustacea, they conduct more slowly than do those of a cold-blooded vertebrate such as the frog, although the latter are of lesser diameter. This fact may be attributed to any of the several differences which exist between the prawn and vertebrate fibres.

SUMMARY

1. The structure of the myelinated fibres of prawns is described, and the homologies of the nucleated sheath which lies between the axon and the fatty layer discussed.

2. The relative thickness of the myelin sheath increases with decrease in total diameter of the fibre along a curve similar in shape to that found in vertebrates and earthworms.

3. Nodes of Ranvier are found in the sheaths of most fibres of a diameter greater than about 13μ.

4. The nodes are similar to those in vertebrate nerves in that the myelin sheath is interrupted at the node.

5. The conduction velocity of fibres in the central nervous system of axon diameter 26μ and total diameter 35μ is between 18 and 23 m. per sec., a rate faster than is found in the "unmyelinated" fibres of similar size in other crustacea.

REFERENCES

ARNELL, N. (1936). *Acta psychiat., Kbh.*, **11**, 5.
ECCLES, J. C., GRANIT, R. & YOUNG, J. Z. (1932). *J. Physiol.* **77**, 23*P*.
FRIEDLÄNDER, B. (1889). *Mitt. zool. Sta. Neapel.* **9**, 205.
GASSER, H. S. & GRUNDFEST, H. (1939). *Amer. J. Physiol.* **127**, 393.
HODGKIN, A. L. (1939). *J. Physiol.* **94**, 560.
JOHNSON, G. E. (1924). *J. comp. Neurol.* **36**, 323.
KUBO, M. & YUGE, A. (1938). *Collected Papers: a tribute to Prof. H. Isikawa.* Kyoto.
MONNIER, A. M. & DUBUISSON, M. (1931). *Arch. int. Physiol.* **34**, 25.
NAGEOTTE, J. (1916). *C.R. Soc. Biol. Paris*, **79**, 259.
PUMPHREY, R. J., SCHMITT, O. H. & YOUNG, J. Z. (1940). *J. Physiol.* **98**, 47.
PUMPHREY, R. J. & YOUNG, J. Z. (1938). *J. exp. Biol.* **15**, 453.
RETZIUS, G. (1888). *Biol. Föreningens förhandlingar*, **1**, 58.
—— (1890). *Biol. Untersuch.*, Neue Folge **1**.
SCHMITT, F. O. & BEAR, R. S. (1936–7). *J. cell. comp. Physiol.* **9**, 261.
—— —— (1939). *Biol. Rev.* **14**, 27.
TAYLOR, G. W. (1940). *J. cell. comp. Physiol.* **15**, 363.
YOUNG, J. Z. (1936). *Proc. Roy. Soc.* B, **121**, 319.

THE BODY TEMPERATURE OF THE FROG

By KENNETH MELLANBY[1]
The University of Sheffield

(*Received* 25 *October* 1940)

(With Four Text-figures)

INTRODUCTION

s well known that poikilothermous animals may have internal body temperatures erent from that of the surrounding air. Their heat of metabolism tends to raise r body temperature, while evaporation of water has the opposite effect. A frog, osed to saturated air, still loses water by evaporation from its skin, because the nal is warmer than the air (Adolph, 1932); the difference in temperature is, wever, only a very small fraction of a degree. In unsaturated air, however, heat by evaporation greatly outweighs heat production by metabolism, and the nal may then be many degrees cooler than the air (Hall & Root, 1930).

This paper describes some experiments showing the effects of evaporation on frog, and measurements of the animal's internal temperatures under a variety conditions.

TECHNIQUE

The internal temperature of the frog was determined using a specially con-ucted mercury thermometer with a small bulb with little capacity for heat; this e readings accurate to 0·1° C. very rapidly. The thermometer was inserted into rectum of the frog. Usually the thermometer was inserted with the bulb at proximately the same temperature as the body of the animal, but even if it were different, its thermal capacity was so small that any error in the result was gligible (i.e. less than 0·1° C.).

When the evaporation from the frog was to be measured, the animal was moved from the water, dried with a duster and squeezed to empty the bladder. his did not always succeed completely, but no urine was passed after the animal d been exposed to air for five minutes.) The frog was weighed to the nearest g. It was then placed in a gauze cage and exposed to a current of air from an dinary electric fan, the speed of the current being varied by altering the speed of motor and the distance of the fan from the cage. This method, though crude, ve air speeds which when tested with an anemometer were found to vary only out 10% over the whole area available to the frog. The best proof of the efficiency the method is given by the uniformity of the results obtained. For accurate work higher air speeds, an apparatus such as that used by Ramsay (1935) is necessary, t for lower velocities the technique used here is quite adequate.

[1] Sorby Research Fellow of the Royal Society.

At appropriate intervals the frog was removed from the cage to determine its temperature and weight. Both these measurements were made within 1 min.

About seventy frogs (*Rana temporaria* L.) were used in these experiments. They were small animals weighing 20 g. or less, and had been starved for some days before being used. The surface area of the skin of these frogs was about 80 sq. cm.

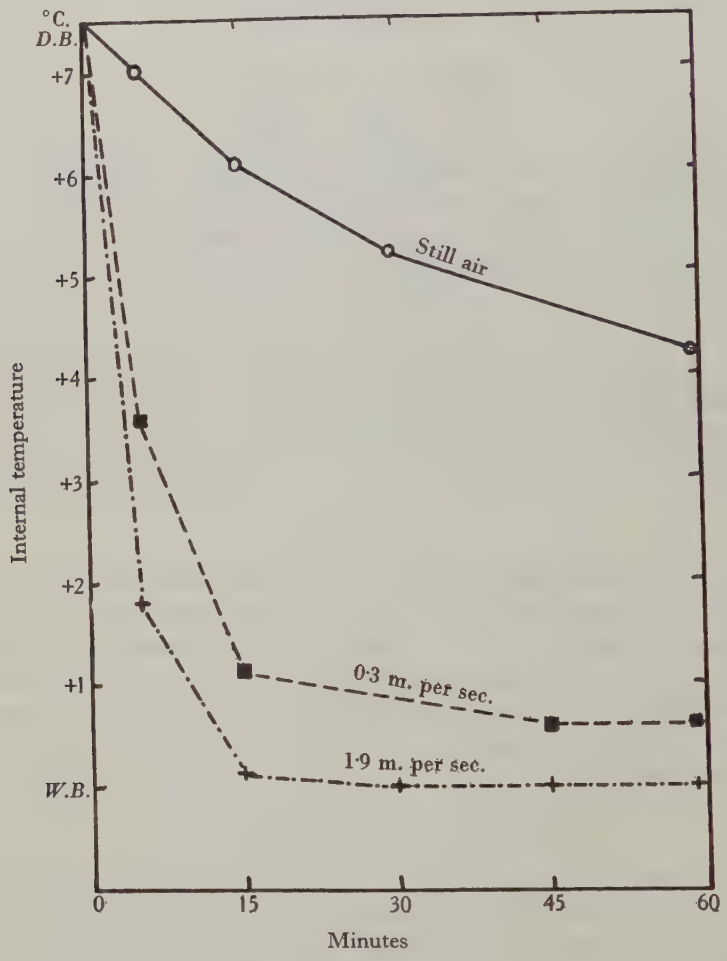


Fig. 1. The internal temperature of frogs exposed to air moving at different speeds. The resu are given in relation to the wet-bulb temperature (*W.B.*). *D.B.* = dry-bulb temperature.

This was determined by removing the skin, floating it on to paper and measuri the area covered. Some previous workers (see Benedict, 1932) have assumed th there is a relation between the weight of a frog and its surface area. This is oft absurd, because starvation and egg-laying can practically halve an animal's weig and desiccation can easily cause a loss of 25 %; starvation or desiccation will hard change the area of the skin.

ie results figured here (Figs. 1–4) are taken from individual frogs. Under r conditions, practically identical results for water loss and internal tempera- ere always obtained, and it appears simpler to give representative examples than average figures. For each of the curves shown, at least ten other les could be given.

RESULTS

(*a*) *The frog's temperature in water*

hen the internal body temperature of a frog which had been immersed in for more than 15 min. was measured, it was never more than 0·1° C. different hat of the water. This result was obtained at many temperatures between 35° C. At the lower temperature, animals previously acclimatized to high ratures went into chill coma (Mellanby, 1940 *a*, *b*); at temperatures over ., heat rigor was frequently obtained (Woodrow & Wigglesworth, 1927).

frog whose body temperature was different from that of the water to which transferred soon assumed the water temperature. This process was complete n 15 min., but within a much shorter period a near approximation was reached. for instance, when an animal with body temperature of 6·0° C. was trans- to water at 28° C., within 5 min. the internal body temperature rose to 26·8° C. rculation of blood, particularly rich in the skin, no doubt assists in this rapid bration.

o matter how much it was stimulated, a frog in water was never found to be ch as 0·1° C. warmer than the surrounding liquid. Metabolic heat must have produced, but its effects on body temperature were very slight.

(*b*) *The frog's temperature in air*

unsaturated air, the frog is considerably cooler than its surroundings. nibian skin is known to offer little resistance to evaporation (Gray, 1928; n, 1939), and the following experiments show how remarkably permeable it is. evaporation is responsible for the very considerable lowering of the animal's temperature.

n one series of experiments, frogs from water at room temperature (21° C.) exposed to air with different velocities and the changes of internal temperature own in Fig. 1, while their loss of weight due to evaporation is shown in Fig. 2. ll air the animal's temperature fell about 3° C. in an hour. In moving air the g temperature was much more rapid amounting to as much as 5° C. in 5 min. moving at a rate of 1·9 m. per sec. With air as rapidly moving as this the frog only 0·1° C. above the wet-bulb temperature (7·4° C. below the dry-bulb erature) within 15 min. The wet-bulb temperature is the lowest temperature n it is physically possible to reach by means of evaporation, so the frog could ery well get much colder. It will be seen that even with air moving as slowly ; m. per sec., the evaporation was sufficient to reduce the internal temperature e animal to within half a degree of the wet-bulb temperature.

At the beginning of each experiment changes in weight of the animals we slightly erratic due to a small uncontrolled production of urine, but it will be see from Fig. 2 that the rate of loss of water soon became remarkably steady. The sma inset on the figure shows the rate of loss after the first 30 min. for four different a velocities.

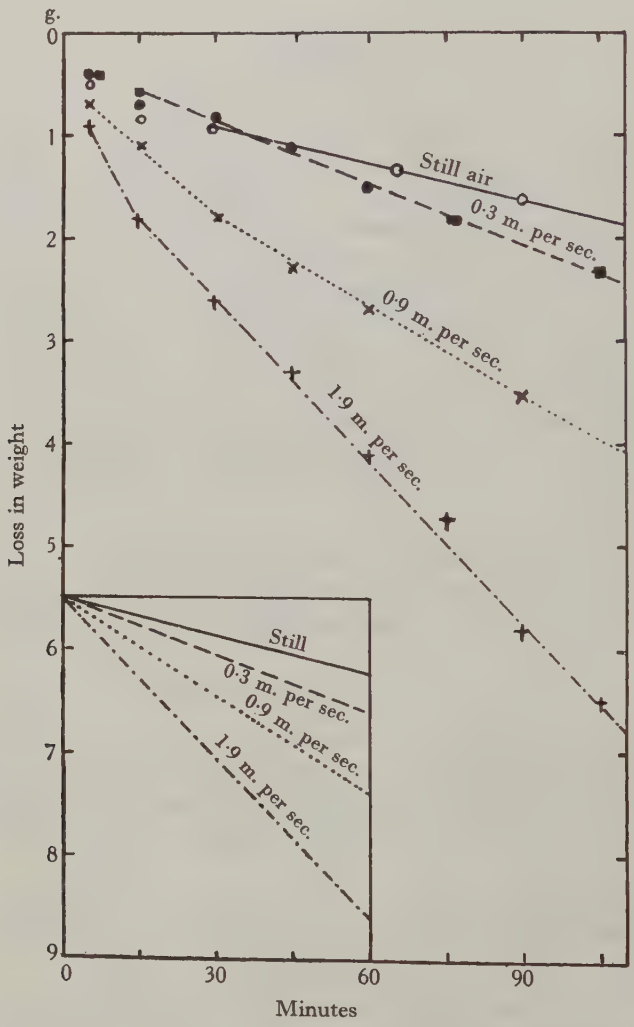
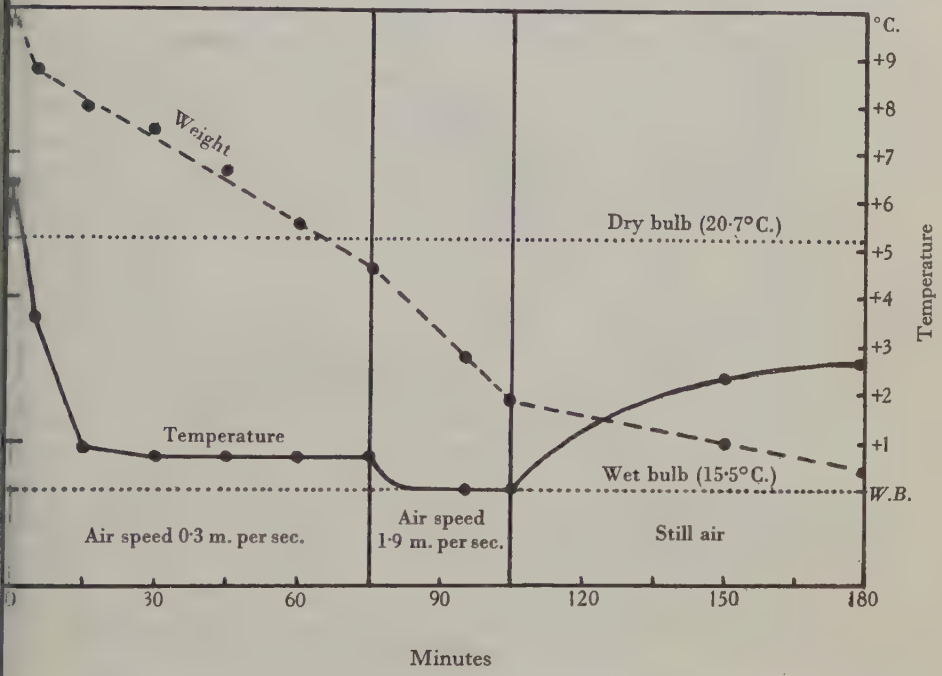


Fig. 2. Loss of weight of frogs exposed to moving air. Inset: rate of loss after first half hour.

The relation between evaporation and body temperature is again shown Fig. 3. Here one frog was exposed first to slowly moving air, then to a more rap current, and finally to still air. Evaporation as measured by change in body weig proceeded moderately rapidly (1·2 g. per hr.) when the air speed was 0·3 m. p sec.; when the air speed was increased to 1·9 m. per sec. the rate of evaporation w doubled and in still air only about 0·3 g. of water was lost during the hour. In t

lly moving air, the body temperature was reduced to within less than a degree ne wet-bulb temperature. The swiftly moving air further reduced the body perature right down to the wet-bulb temperature and then in still air the frog me nearly 3° warmer.

Even after it was dead the frog continued to lose water at an equally rapid rate. ig. 4 a small frog weighing at the start 12·2 g. was desiccated by being exposed current of 0·3 m. per sec. for 24 hr. The animal lost water at a rate of about



3. The internal temperature and loss in weight of a frog exposed to different air velocities.

per hr., and this loss proved fatal in about 2½ hr. However, the rate of loss was ntained steadily for 6 hr. until the animal had lost 50% of its original body ght. After this the loss in weight fell very considerably, so that during the final ur. the animal only lost ½ g. and finished by being almost completely desiccated, ng lost 74% of its original weight. It should be noted that to the touch the skin he animal appeared quite dry, even some time before it died although it was ng water by evaporation at this very considerable speed. During the first few utes this frog's internal temperature fell 6·5° C. to just above the wet-bulb perature. So long as the rapid evaporation was maintained (i.e. for about 6 hr.) internal temperature of the animal remained at this low level, but during the er part of the experiment when the desiccated animal was losing water slowly, internal temperature rose and at the end of 24 hr. had practically reached air

temperature. The fact that this animal lost water as rapidly after death as whe alive, means that in comparison with the loss from the skin, evaporation durin respiration must have been negligible.

DISCUSSION

As the internal temperature of a frog exposed to moving air is the same as th wet-bulb temperature, evaporation must be taking place as rapidly as is physicall possible. Even if the animal had no skin, water could not be lost more rapidly. It of interest to compare this process with the speed of water uptake through the ski

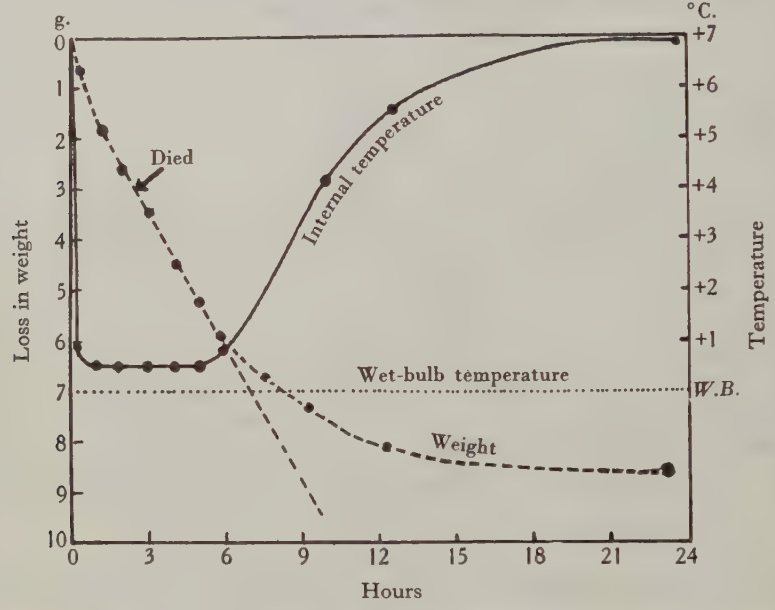


Fig. 4. The internal temperature and loss in weight of a frog desiccated until death and for many hours after death.

of the frog. Adolph (1933) states that "it may be noted that the most rapid desic tion by a current of dry air caused the frog to lose water less rapidly than the sa frog gained water when put into water again", but this statement is not corre The frog exposed to an air current of 1·9 m. per sec. (see Fig. 2) lost 3·2 g. of wa per hour but only absorbed 1·6 g. per hr. when returned to water. This rate of crease is as rapid as was ever found by Adolph (the frog weighed 21 g. undesiccat and so gained nearly 8% and lost almost 16% in an hour), but with more ra currents and drier air considerably greater losses of water were obtained over sh periods. The limit to the rate of loss is probably governed by the speed with wh water reaches the skin and not by its permeability. The comparative slowness uptake of water by osmosis, notwithstanding the difference of osmotic pressure about 4 atm., must be due to its "stagnation" within the thickness (70μ) of the sk

When we say that the internal temperature of a frog exposed to moving ai the same as the wet-bulb temperature, it is obvious that this is only an approxi

The salts dissolved in the body fluids must tend to prevent evaporation and to up the temperature. However, water evaporates from a solution of equivalent gth very nearly as fast as from distilled water unless the air is almost saturated, nder most experimental conditions the effect of the substances dissolved will actically negligible. Then the heat of metabolism of the frog will tend to raise ternal body temperature. It may be of some interest to give an idea of the itude of the two processes of heat production by metabolism and heat loss aporation. At 20° C. a 20 g. frog will produce approximately 2 mg. CO_2 r. (Vernon, 1895); this means that about 6 cal. will be produced. This frog ose 3·2 g. of water by evaporation in an hour. The evaporation will absorb 2000 cal. (Mellanby, 1932). This comparison makes it obvious that in dry air eat gained by metabolism is negligible compared with the enormous loss due aporation. It is not always realized how low is the rate of metabolism of lotherms. Even when they are warmed to 37° C., at which temperature their olism reaches a maximum, reptiles and amphibians still have a metabolic rate about 20% of that of a mammal of similar size (Benedict, 1932).

SUMMARY

nder most conditions the amount of metabolic heat produced by a frog is so that the animal behaves like a non-living system and its temperature is con-d by external physical conditions.

he frog's skin is so permeable that when the animal is exposed to moderately ly moving air (1 m. per sec. or over) evaporation reduces its internal tempera-o the wet-bulb temperature.

bout 25% of the frog's weight may be lost by evaporation before death ensues. death water continues to evaporate at the same rapid rate until 50% of the al's weight is lost.

REFERENCES

Adolph, E. F. (1932). *Biol. Bull. Wood's Hole*, **62**, 112–25.
—— (1933). *Biol. Rev.* **8**, 224–40.
Benedict, F. G. (1932). *The Physiology of Large Reptiles*. Washington.
Gray, J. (1928). *Brit. J. exp. Biol.* **6**, 26–31.
Hall, F. G. & Root, R. W. (1930). *Biol. Bull. Wood's Hole*, **58**, 52–8.
Krogh, A. (1939). *Osmotic Regulation in Aquatic Animals*. Cambridge.
Mellanby, K. (1932). *J. exp. Biol.* **9**, 222–31.
—— (1940*a*). *Nature, Lond.*, **146**, 165.
—— (1940*b*). *J. Physiol.* **98**, 27*P*.
Ramsay, J. A. (1935). *J. exp. Biol.* **12**, 355–72.
Vernon, H. M. (1895). *J. Physiol.* **17**, 217–92.
Woodrow, C. E. & Wigglesworth, V. B. (1927). *Biochem. J.* **21**, 812–14.

ON OVIPOSITION, OLFACTORY CONDITIONING AND HOST SELECTION IN *RHIZOPERTHA DOMINICA* FAB. (INSECTA, COLEOPTERA)

By A. C. CROMBIE

From the Zoological Laboratory, Cambridge

(*Received* 1 *November* 1940)

(With One Text-figure)

I. INTRODUCTION

The hypothesis that olfactory conditioning may influence the production of bio logical races, by the differentiation of a species into groups of individuals, eac associated with a particular food, has been suggested by Imms (1931) and b Uvarov (1932). Thorpe & Jones (1937) have shown that such conditioning ca actually occur and have stressed its possible importance as an isolating facto Preference for different species of hosts, as food or for oviposition, by differen biological races, coupled with or even without a prevailing avoidance of cross mating, would tend to preserve subsequent genetic changes by preventing them from spreading beyond the race in which they arose. In other words the so-calle "Sewall-Wright effect" of conservation of random, non-adaptive change migh become operative. Indeed, a number of earlier writers have claimed that biologic races do afford instances of species in the actual process of formation.

As the occurrence of biological races in insects and other animals has bee reviewed by Thorpe (1930, 1939*b*) there is no need for further discussion her Recently, Thorpe & Jones (1937) and Thorpe (1938) have conducted conditionin experiments with the ichneumon, *Nemeritis canescens* (Grav.), an endoparasite o the larva of the moth, *Ephestia kühniella* (Zell.). The *Nemeritis* developed a positiv response in an olfactometer to the odour of an abnormal host, viz. the larva of th moth, *Melliphora grisella* (F.), when it was reared on that host or exposed to th odour of the latter in a conditioning apparatus. Further, by using similar method *Nemeritis* has been induced to develop tolerance to cedar-wood oil, while *Drosophi melanogaster* Mg. (Thorpe, 1939*a*), when reared upon food impregnated wit peppermint oil, developed a positive response to the odour of that substance: bot *Nemeritis* and *Drosophila* are normally repelled by the substances mentioned.

Most of the insects used in experiments dealing with host selection have bee either monophagous or only slightly polyphagous in habit. It would seem unlike that a highly polyphagous animal would develop strong attraction to individual ho species, and this conclusion appears to be indicated in the case of the "weevil

hus quadrimaculatus (Larson, 1927). These observations, however, failed to lish (*a*) whether chemical stimuli enable the *Bruchus* to find its host, and (*b*) the played by such physical variables as shape, size, surface texture, etc. It did a predilection for smooth, well-filled seeds, and this could have masked any ical conditioning effect there may have been. In the present work another ly polyphagous insect was used, viz. the "lesser grain borer" or "Australian t weevil", *Rhizopertha dominica* (Fab.). It is known to infest grains of all kinds ther stored products. A full account of the biology of *Rhizopertha dominica* en by Potter (1935), and further information by Barnes & Grove (1916) and enden (1911). At 30° C. and an approximate relative humidity of 70% the ing period is about 7 days or less. An active larva emerges, crawls away from pot where it was hatched, burrows into a grain—in the case of undamaged t through the embryo—and emerges as an adult in about 4–5 weeks. The total istory takes therefore about 5–6 weeks. Larvae and adults live on the same . The adults appear ready to lay eggs about a week after emerging from the in which they developed, but the age at which they do so probably depends hen they are fertilized.

II. TECHNIQUE

s there are no external differences between adult males and females, they were nguished by squeezing each animal until the tip of its genitalia appeared. The aying capabilities of squeezed females was found not to be impaired nor length fe shortened. The sex ratio was approximately unity. The animals used for e experiments were, unless otherwise stated, for the most part from 2–3 weeks t the beginning of each experiment. As the males copulate at every opportunity of the females will be fertilized. They are considered as being so.

Where Petri dishes were used their floors were covered with a layer of plaster aris to enable the animals to walk easily. They are almost helpless on glass.

The olfactometer used was of the type described by Thorpe (1937, 1938) which sentially a Y-shaped tube. The odoriferous substance to be tested is put in one and the other left empty. A vacuum pump attached to a tube from the stem ne Y draws a current of air down through the two arms; the strengths of the ents in each arm being made equal by flowmeters. For these experiments pparatus was used horizontally and the glass ground inside on the bottom half ne tube. This enabled the animals to walk. To facilitate grinding the apparatus T-shaped. The animals were put in the base of the stem and attracted down it light. On reaching the division of the two arms they were forced to make oice. Those going each way were caught in containers, counted, and the results xed out statistically. The apparatus was washed at intervals with water and nol, and the "bait" changed from arm to arm to eliminate errors due to any bias ne tube.

All experiments were performed at 30° C. and a relative humidity of approxi-ely 70%, and, except for those with the olfactometer or where otherwise stated, arkness.

III. THE CHOICE OF OVIPOSITION SITE

From preliminary experiments in which the insects were given the choice f oviposition of the various kinds of seeds mentioned in § VII, it became evident th seeds with crevices, regardless of size or general shape, were the most favoure For example, eggs were laid in wheat and barley under the test or between t cotyledons, in maize under the loose test at the apex, and underneath the flat surfa of lentils. Few or no eggs were laid on smooth seeds such as beans or peas. T obvious differences between the various seeds used are size, shape, texture, odou taste and visibility. The influence of these characteristics were investigated.

Size. In eight dishes five balls of plaster of Paris of 0·25 cm. diameter we arranged alternately with five similar balls of 1 cm. diameter, while in seven oth dishes five balls of 0·5 cm. diameter were arranged alternately with five others 2 cm. diameter. The range of size chosen covered that of the seeds used in t preliminary experiments. The balls all had small crevices in which eggs were oft laid. Seven males and seven females were put into each dish, and the dish examined for eggs after being left undisturbed for 7 days. The total numbers eggs laid in the first set of dishes were fifty in the larger and forty-six in the small balls, and in the second set fifty in the larger and forty in the smaller balls. For t fifteen dishes altogether 53·8% of the eggs were laid in the large and 46·2% in t smaller balls. As the difference between 53·8 and 50% is not greater than twice t standard error of $\pm 3{\cdot}66\%$, this difference cannot be regarded as significant. Als there was no consistent preference for larger or smaller balls throughout the fifte dishes. Therefore, unlike *Trichogramma*, which Salt (1935) found had a defini preference for larger hosts over small, *Rhizopertha* has no preference for size host within this range.

Shape. A number of objects of various shapes were put into each of seven dish both objects and the floors of the dishes being made of plaster of Paris mixed wi wholemeal flour. The latter provides the odour of food as an inducement to ov position. The objects used may be described as follows: A, square pieces of pap ($0{\cdot}5 \times 0{\cdot}5$ cm.) fixed to the floor of the dish with wet plaster; B, similar pieces paper similarly fixed to plaster hemispheres of 2 cm. diameter; C, plaster hem spheres of 1 cm. diameter; D, plaster spheres of 1 cm. diameter each with two hol of 2 mm. diameter; E, ten similar holes made in the plaster floor of each dish. Fi of each of objects A, B, C and D were arranged in groups each of which contain one object of each shape, each group having beside it two holes (E) in the floor the dish. Ten males and ten females were put into each dish, and the latter examin for eggs after 7 days. The total numbers of eggs laid in each kind of object we 439 in A, 427 in B, 108 in C, 29 in D, 31 in E, and 40 loose about the dishes. T proportion laid in each kind of object was much the same in each dish. The anima preferred the situation provided by paper fixed to plaster, eggs being laid here the case of A and B, under the flat bottoms of hemispheres in the case of C and some extent of B, and in the holes in the case of D and E. They seem indiffere as to where the paper was fixed, or the holes made. The proportion of eggs la

r paper fixed to plaster, under plaster hemispheres, and in holes in plaster res, may be compared with those laid in the preliminary experiments under the in maize, under lentils, and on pearl barley or buckwheat, respectively. rence for crevices is definite.

exture. In each of six dishes two groups of five rough objects were alternated two groups of five smooth objects of the same size and shape. The rough ts used were plaster balls and stones, the smooth objects lead shot, glass beads broken glass, only one kind of object of each class, rough or smooth, being used ach experiment. Their size did not extend beyond that of the objects in the r experiment with a choice of sizes. Seven males and seven females were put each dish, and the latter examined for eggs after 7 days. In the six dishes the number of eggs laid among the rough objects was sixty-six, or 86·7%, while eleven were laid among the smooth objects. This shows that the animals prefer ts with rough surfaces for oviposition rather than those with smooth surfaces. rom these experiments it is concluded that for oviposition *Rhizopertha* is ferent to the size of objects between 0·25 and 2 cm. diameter, prefers rough ces to smooth and has a strong liking for crevices. Ullyett (1936) found that *oplectron* ceased to examine pith cubes after the sharp edge was found with the nae, but examination of smooth round gelatine capsules continued. *Rhizopertha* lly examines with its palps any object which it encounters. If holes or crevices ound the examination is prolonged, if not it is usually brief.

ffect of food. Seven series of experiments were carried out: in each series ty Petri dishes were used, each dish containing five male and five female *opertha*: thus 1400 insects were used in all. The eggs laid in each dish were ted and removed daily for a period of 5 days. In series A, the control, ten amaged wheat grains were placed in each dish. The test covering the embryo wheat grain is not attached to the underlying tissue as it is over the cotyledons. e it was slit along one side in each grain, thus providing a crevice for oviposition. eries B ten artificial grains, similar in size and shape to the natural objects, made by mixing ground wheat with plaster of Paris in the proportion of 1 : 3. ach of these a piece of paper 5 mm. square was fixed with wet plaster near one to imitate the test over the embryo in wheat. In series C ten similar artificial ns without paper attached were placed in each dish. In series D each dish ained ten artificial grains of similar shape, each with paper attached as in series B, composed of plaster of Paris alone, while in series E ten artificial grains composed laster of Paris alone but without paper were placed in each dish. In series F G no objects were placed in the dish; but in series F their floors were composed round wheat mixed with plaster of Paris as for the artificial grains, while in s G the floors of the dishes were of plain plaster of Paris as in all the other dishes. number of eggs laid during each series of experiments is given in Table 1. artificial grains (A) containing food and with paper attached to them induced females to lay as many eggs per day as did the wheat grains (B) themselves, are therefore perfect "experimental hosts". But there was a sharp drop in the of oviposition when the artificial grains either had no paper (C) attached even

though they did contain food, or had paper attached (D) but contained no food When they had no paper attached the presence of food in their make up (C induced the females to lay eggs at a greater rate than when they contained n food (D), while on the other hand when they contained no food the presence o paper made little or no difference (compare D and E). Likewise, when there were no objects at all in the dishes with the animals, the presence of food in the floo made little difference to their rate of oviposition (compare F and G). It is to b noticed that the animals were starving in all the series of dishes. *Rhizopertha* cannot attack undamaged grain, and the wholemeal flour present in the artificia grains containing it could not be eaten.

Table 1. *The stimuli inducing oviposition*

Series	Eggs per ♀ per day			Total eggs (in 20 dishes) in 5 days
	Max.	Min.	Mean	
A. Wheat grains	2·91	1·04	2·01	1005
B. False grains of plaster and wheat, with paper	3·20	1·11	2·17	1085
C. As B, but without paper	0·62	0·28	0·438	219
D. False grains of plain plaster, with paper	0·18	0·06	0·104	52
E. As D, but without paper	0·16	0·03	0·098	49
F. Dish floor of plaster and wheat	0·03	0	0·016	8
G. Dish floor of plain plaster	0·05	0	0·01	5

In series A, B and C eggs were laid in twenty-five out of thirty dishes by th second day of the experiment whereas no eggs were laid in series D and E until th third day and then in two dishes only. In series F and G eggs were laid in two dishe on the fifth day. By the fourth day all the dishes in series A, B and C containe eggs whereas this was not achieved in series D and E until the fifth day and i series F and G not at all. It appears therefore that in the absence of both the odou of food and of suitable tactile stimulation, the full oviposition response is not evoke and the animal may be said to exercise restraint. In this behaviour *Rhizopertha* resembles *Pimpla instigator*, which will not oviposit unless stimulated by both th odour of its host's blood and suitable tactile sensation (Picard, 1922).

Sight. As discrimination between objects of various shapes was exercised i complete darkness the sense or sight does not appear to be necessary for thi purpose. But if *Rhizopertha* becomes dispersed by flying freely, the sense of sigh may be of importance. That they are photopositive is shown by their behaviour i the olfactometer.

The visibility of objects was tested in the following way. The tops of thre transparent celluloid capsules, 0·5 cm. in diameter, were stood in a circle on smoot white paper. Alternating with them were three similar capsules which had bee blackened on the inside with Indian ink. All six capsules were, therefore, of simila external texture. A circle 1·5 cm. in diameter was drawn around each of the point where a capsule was standing. For each experiment, after placing four insects i

centre of the circle of capsules the whole was covered with the lid of a Petri
The results of observations conducted for a period of 1 hr., using sixteen
s and sixteen females, are expressed in Table 2. The following records were
(1) when an insect made contact with a capsule and ceased to move; (2) when
proached a circle tangentially and then turned at a right angle as if it had seen
apsule inside; (3) when it walked through a circle without stopping; and (4) the
ber of contacts made of over 15 sec. duration as a control. The observations
repeated using eight females and eight males in which both eyes had been
ened with ivory black water colour pigment. They were examined before and
each experiment.

Table 2. *Visibility of objects*

		(1) No. of contacts	(2) No. of deflexions at right angles	(3) No. of times circle traversed
16 normal ♀♀:	Transparent	74	6	55
	Black	100	38	19
8 blinded ♀♀:	Transparent	55	5	44
	Black	64	8	47

When a normal *Rhizopertha* encounters a black capsule it approaches to within
t 5 mm. of it, stops, raises its head and waves its antennae. Afterwards it
s nearer and taps the capsule with the tips of the palps. With transparent
ules the insects often approach to within 1 mm. or even reach them before
ping. They attempt to climb more often on to the black capsule while they
uently try to push straight through transparent capsules. Control animals with
blackened usually collide with capsules before stopping. From Table 2 it will
parent that with the normal animals the number of contacts (1) and of deflexions
ght angles towards a capsule (2) is very much greater in the case of black than
e transparent capsules, while the circle is traversed without contact with the cap-
s (3) more frequently in the case of the transparent than of the black capsules.
hermore, contacts with the black capsules are of greater duration than those
the transparent capsules, the longest being 6 min. with the black and only
ec. with the transparent capsules. None of these statements hold true for the
ded animals. The latter tended to remain in contact with both sorts of capsules,
ongest contacts being 3 and 4 min. with transparent and black capsules respec-
y. The results of identical experiments with males closely resembled those with
females. It is concluded therefore that black capsules proved more attractive
hizopertha because they could be more readily seen. Ullyett found that *Micro-*
ron continued to examine opaque capsules filled with brown paper for a longer
than they did transparent capsules.

IV. THE HOST SELECTION PRINCIPLE

The "Host Selection Principle" of Walsh and Hopkins states that an adult fema may be attracted for oviposition to the particular species on which she fed a larva (see Craighead, 1921, 1923). *Rhizopertha*, bred originally from barley, w reared for two generations on the following kinds of seeds: wheat, barley, buckwhe maize and haricot beans. The following experiments were then carried out. In t first experiment five of each of the above seeds were placed in each of a number Petri dishes. Then five males and five females reared from one of these kinds seeds were introduced into each dish, where they had the choice for ovipositi of the seed in which they had been reared and of the remaining four other kinds seed. Nine dishes were used in all, and except that there was only one dish wi animals reared in haricot bean, each pair of dishes contained animals reared fro one particular kind of seed. All of the seeds were split by tapping. The eggs la in each dish were counted after 10 days and it was found that the numbers of eg laid in the kind of seed from which the animals had been reared were not uniform greater than those laid in other kinds.

The next experiment was designed to eliminate differences in shape, size ar the presence of crevices in the seeds. Hemispheres of plaster of Paris mixed wi ground seeds of the various kinds were made of the same size (0·5 cm. diam and shape, together with similar hemispheres of plain plaster. A piece of pap $\frac{1}{2}$ cm. square was fixed with wet plaster to the top of each. They differed now on in smell and taste. Using these the last experiment was repeated. Again there w no marked predilection for oviposition in the food in which they had been reare Of the five hemispheres of each kind never more than three of a particular kir were used for oviposition in any dish, and often only one or two: the insects wer therefore, not forced by lack of space to lay in all the hemispheres. As many eg were laid on the plain hemispheres as on those containing food and this m indicate that the sense of taste was not employed in the selection of oviposition sit

A third experiment with the same set up was performed in which one particul type of seed in each dish was made most favourable by ensuring that it had crevic and that none of the others had. Thus to make them favourable the loose test ov the embryo of the wheat grains was slit along one side; barley grains were chos for loose tests; haricot beans chosen for broken tests; maize chosen for the presen of test at the apex. To make them unfavourable wheat and buckwheat were le undamaged; the test of barley removed; haricot beans chosen for unbroken test and the test at the apex of maize scraped off. Five males and five females, rear in barley, were placed into each dish and the eggs counted after 10 days. Table shows that the relative numbers of eggs laid in each kind of seed are clea dependent on the presence of crevices. Eight dishes were used in all, and the figu in each of the columns in Table 3 represent the total of two dishes in each ca The sense of touch seems, therefore, to prevail in the selection of the ovipositi site once the animal is within an environment smelling of food. Likewise *Trich gramma evanescens* is not influenced in its choice of oviposition site by the host

h it was reared. With unparasitized hosts its choice appears to be determined ie relative sizes of the hosts present, which in darkness it appreciates presumably ictile sensations.

n a final experiment, using the same set up, eggs laid by females reared in the rent seeds were placed in Petri dishes with two grains each of wheat, barley, wheat and maize. The grains were crushed or split so that differences in hard- of test would not interfere with the choice. The dishes were examined for e at the end of a week, by which time most of the eggs had hatched. The larvae ed no predilection for entering the kind of grain in which their parents had reared.

Table 3. *Host selection principle*

ach of the figures in the four columns represent the total number of eggs laid in a pair of dishes each of the kinds of seeds present. In each pair of dishes one particular seed was made rable (see text) as indicated.

Each dish contained five of each of the following seeds	Wheat favourable	Barley favourable	Maize favourable	Haricot bean favourable
Wheat	44	3	0	5
Barley	2	71	0	2
Buckwheat	2	5	1	0
Maize	3	7	48	3
Haricot bean	0	0	0	57

V. LOCATION AND FUNCTION OF CHEMORECEPTORS

A review of the experimental evidence supporting the various views about the tion and function of chemoreceptors in insects has been given by Marshall 5). An attempt was made to elucidate some of the problems concerned as rds *Rhizopertha*.

Discovery of food. It was essential first to obtain precise knowledge both of the aviour of the animal on encountering food, and of the influence of different itional states on this behaviour. A number of animals were starved for 1 day placed in a dish with hemispheres made of plain plaster and of plaster mixed barley, maize, wheat and buckwheat, in equal numbers. The animals were erved under a binocular microscope, and a record kept of the number of visits feeding reactions with each hemisphere. A total of 7 hr. observation using gether seventy animals showed that the reaction to each of these foods after ay's starvation was to extrude the mandibles and gnaw. This feeding response never observed when animals encountered hemispheres containing food. In e dishes left overnight, twenty-eight out of thirty animals were found next rning gnawing the food hemispheres. In the proximity of an object diffusing odour of food the head is held up with antennae waving and palps trembling, le searching movements are shown until the object is encountered. After a pre- nary examination with the palps the feeding response follows, and the object be tunnelled into. Little interest is shown in objects not containing food

unless they are provided with crevices or holes, in which case the latter are subjecte to an exhaustive examination. No difference in the behaviour of males and female was observed.

Animals in different nutritional states show certain differences in behaviou When well fed, although plain plaster hemispheres never elicited the feeding response little more interest was shown in those containing food. The animals spent most o their time examining holes. After 7 days starvation, hemispheres containing foo were attacked to the exclusion of those of plain plaster. If only the latter wer available, they elicited, however, the feeding response, i.e. they were gnawed. Suc behaviour was never observed in less hungry animals. Nash (1930) found that in th tsetse-fly (*Glossina*), although the antennae usually call forth the probing response an antennaless animals rarely show it, a very hungry fly will probe in the absence o antennae. Minnich (1922, 1929) also found that with *Calliphora* and *Pyrame* during periods of starvation on a water diet the threshold of response to sugars falls For saccharose solutions the threshold falls suddenly after several days, and h suggests that this fall may be due to a fall of inhibition by the central nervou system.

A *Rhizopertha* beetle on encountering a food hemisphere may touch it with it maxillary palps and no other part of the body, then begin gnawing as a reaction t food. There are no visual or tactile differences between the food and plain hemi spheres, therefore the animal must either have smelt the food or tasted it throug the palps. To determine the location of the chemoreceptors various parts of th body were removed or lacquered. A solution of lac in absolute alcohol was use and applied, while the animals were held with forceps, with a fine glass rod unde a binocular microscope. It runs along the organ concerned and envelopes it. Th lacquer may remain for several weeks, but animals were examined before and afte each experiment. Most of the mutilated and lacquered animals were still alive afte 2 months. It was found that absolute alcohol alone had no effect on behaviou After treatment the animals were given a day to recover and starved for another da before the experiment. Their nutritional state was thus standardized. For eac observation several males or females were placed into a Petri dish with two hemi spheres each of plain plaster and of wheat and plaster and watched for an hour unde a binocular microscope. A record was kept of the number of visits to the differen kinds of hemispheres during which the feeding response was shown, as well as o the total number of visits to each kind. It was found that the plain plaster hemi spheres failed to elicit the feeding response, although those containing wheat di when the following organs or combinations of organs were removed: maxillar palps; maxillary and labial palps; antennal clubs; and maxillary and labial palp and antennae. The same behaviour resulted when the legs alone were lacquered But when the legs were lacquered and the antennal clubs at the same time lacquere or removed, the plain plaster hemispheres as well as those containing wheat calle forth the feeding response and were, without distinction, gnawed. Thus with a tot of fifteen animals treated in the last-mentioned fashion the feeding response followe in eight out of twenty-three visits to wheat and plaster, and in seven out of twenty-tw

s to plain plaster, hemispheres. This was confirmed with ten animals which no antennae, legs lacquered and with palps removed as well. The feeding response wed in thirteen out of twenty-seven visits to wheat and plaster, and in thirteen of twenty-nine visits to plain plaster, hemispheres. If, while the antennal clubs removed or lacquered, only the fore-legs, or the fore-tibiae and tarsi were uered, then the plain plaster hemispheres were also gnawed, but the preference hose containing wheat was not altogether lost. Thus with twenty-one animals no antennal club and fore-legs alone lacquered the feeding response followed ghteen out of thirty-six visits to wheat and plaster hemispheres and in six out irty-one visits to those of plain plaster; and with twenty animals with antennae fore-tibiae and tarsi lacquered the feeding response followed in twenty-three of sixty-seven visits to wheat and plaster, and in three out of fifty-seven visits lain plaster, hemispheres. And in dishes left overnight eleven out of twelve als with no antennae and fore-legs lacquered were found next morning on the ispheres containing wheat. Chemoreceptors must therefore be present on the nd and third pairs of legs as well as on the first pair. Further, whereas when one of the antennal clubs was left untreated, the other and all the legs being uered, the plain plaster hemispheres were never gnawed, when one fore-tibia tarsus alone was left functional they were gnawed in one visit out of twenty-eight e those of wheat and plaster were gnawed in seventeen out of twenty-nine visits. se remarks apply equally to both sexes. These observations show that the al does not find its food by taste, and, since animals with palps present but nnae removed and legs lacquered, cannot distinguish between plain plaster and at and plaster hemispheres, they finally rule out the palps as chemoreceptors. se are probably chiefly concerned with touch. Experimental evidence points efore to the antennae and legs—in the latter chiefly the fore-tibiae and tarsi—e sites of the chemoreceptors in both males and females. By similar methods ott (1927) and others have shown that chemoreceptors may be present on the nnae and other parts of the body in Coleoptera, while Minnich (1922; 1929) established their presence on the tarsi of various butterflies and flies.

In *Rhizopertha* the feeding response seems normally to be elicited by olfactory uli. But when the olfactory receptors are put out of action this response seems llow tactile stimuli: plain plaster hemispheres are gnawed, crevices and holes g favourite places. Brecher (1929) has observed that the removal of the antennae he cockroach lowers the threshold for stimulation of the photoreceptors. In *dnius* the feeding reaction is normally elicited by warmth and smell, but removal e antennae lowers the threshold of stimulation by sight, so that moving objects me irresistibly attractive (Wigglesworth & Gillett, 1934). If the eyes are kened as well, vibrations elicit the feeding response. It may be that *Rhizopertha* ormally inhibited from gnawing substances without an appropriate smell, but n the olfactory organs are put out of action or with extreme hunger, the threshold the feeding response to tactile stimulation falls because of the fall of the central bition.

Recognition of the opposite sex. In an olfactometer males are not attracted by

smell to a "bait" of twenty females. To this question of recognition of the opposit sex are linked those of sexual selection and of mating barriers between biologica races (Richards, 1927), and accordingly the behaviour of the sexes towards eac other at closer quarters was observed. Males were marked with a spot of whit paint on the elytra, and for each observation three of each sex were watche together for an hour in a dish. The meetings between male and female, and mal and male, and the attempts to mate, were recorded. In another series the observa tions were repeated with legs and antennae lacquered. It was found that even wit normal males attempts at mating with other males sometimes occurred. Thus i four observations attempts at mating were: males with females = 29, males wit males = 4. But when both antennae and legs are lacquered in the males all dis crimination for mating on their part between the two sexes is lost: in four observa tions attempts at mating of males with females = 13, males with males = 13. Bu when either legs or antennae are left unlacquered discrimination remains un diminished. In two observations when the antennae alone of the males wer lacquered, attempts at mating of males with females = 13, male with male = 1; an similarly when the legs alone of the males were lacquered, attempts at mating o males with females = 12, males with males = 0. As the male is the active partner i mating, one would not expect the lacquering of the female's legs and antennae t affect the number of attempts at mating with the same sex: in two observation attempts at mating of males with females = 13, males with males = 2. Females neve attempt to mate with each other. But if the legs and antennae of both males an females are lacquered then discrimination is lost as before: in two observation attempts at mating of males with females = 7, males with males = 6. The olfactor receptors for recognizing the opposite sex as well as food are therefore to be foun on these organs. Probably they are the same, as Valentine (1931) believed them t be in *Tenebrio*. It appears that the female odour neither arouses the male to g searching for her as it does with *Tenebrio* (Valentine, 1931), nor elicits the matin reflex as it does with the silkworm moth, *Bombyx mori* (Kellogg, 1907). There is n attraction between the sexes until they meet, and then they recognize each othe by smell. This agrees with what Hunter & Hinds (1905) found in the cotton-bo weevil.

VI. OLFACTOMETER EXPERIMENTS

Responses to natural foods. The responses of the insects to various substanc were determined by means of an olfactometer. In these experiments each individu animal ran through the apparatus about three times. The results expressed i Table 4 indicate that both males and females were attracted to clean wheat. The were starved for 2 days before the experiment. But the percentage of the anima going to the arm of the olfactometer containing the "bait" was, although significant different from that to be expected were chance the only operative factor (i.e. 50% not very high. This was a feature of all the experiments performed in the olfact meter with *Rhizopertha* and renders the results less reliable than they would ha been had the responses of the animal been more definite (Hoskins & Craig, 1934

homogeneity of the various series of observations was tested by calculating χ^2 values, which are given in the tables concerned. If χ^2 does not exceed the corresponding to a probability (p) = 0·05 for $n-1$ degrees of freedom (where mber of observations), then the samples may be considered as reasonably geneous. The 5 % values of χ^2 may be found in the tables of Fisher & Yates). The standard errors were looked up in the tables of Purewal & Rao (1936). n experiment was now performed in which 100 males and 100 females were apart but fed for 7 days, and the attraction of each towards a bait consisting nty of the opposite sex tested in an olfactometer. Out of 253 males 124 = 49 % 3·14 %) went to the arm containing the females; while out of 258 females 53·1 % (S.E. = ± 3·11 %) went towards the males. There was therefore no tion between the sexes in an olfactometer and they could be used together e experiments. This avoided the labour of sexing large numbers of animals ueezing.

Table 4. *Responses to wheat*

obs.	Wheat	Blank	Total	No. insects	S.E.	χ^2
5	271 = 61·9 %	167	438	150 ♂♂	± 2·39 %	74·82
7	273 = 60·9 %	175	448	150 ♀♀	± 2·36 %	30·70

he intensities of attraction of animals reared in various natural foods to the in which they were reared were now determined, and compared with the sities of attraction to the same foods of animals reared in wheat. The foods erned were barley, oats and maize and the animals represented the second ration reared from them in each case. The "bait" consisted of roughly 2 c.c. e crushed grain. The insects were from 2 to 3 weeks old and before testing were ed for 1 day after removal from the food in which they had been reared. As expected from the experiments described in § IV, the percentage of animals cted towards a certain food was no greater for animals which had been reared than for other animals. Thus, using animals reared in wheat, out of 886, = 61·4 % were attracted to wheat; out of 270, 167 = 61·9 % were attracted to y (S.E. = ± 3·04 %); out of 253, 157 = 62·1 % were attracted to oats (S.E. = 14 %); and out of 310, 184 = 59·4 % were attracted to maize (S.E. = ± 2·84 %). pared with these figures, with insects reared in barley; out of 678, 409 = 60·3 % attracted to barley (S.E. = ± 1·92 %); with insects reared in oats, out of 277, = 61·4 % were attracted to oats (S.E. = ± 3·00 %); with insects reared in maize, of 311, 187 = 60·1 % were attracted to maize (S.E. = ± 2·84 %). Further, when insects reared in maize were given the choice of maize and wheat in an olfacto- r, out of 520 insects running through the apparatus, 272 = 52·3 % went to the e arm. As the standard error is equal to ± 2·19 % this percentage is not ficantly different from 50 %.

Responses to peppermint. These experiments were designed to determine whether responses of the insect to the odour of peppermint could be affected by sub- ng it to that odour during adult life. As a control, the responses to peppermint

of animals reared in barley were determined. Peppermint[1] was used as a bait in the form of four drops of 10% solution in alcohol on 4 sq. cm. of no. 1 Whatman filter paper, and allowed to dry. Table 5A shows that 62% of the animals were repelled.

A number of animals of both sexes were now kept in a "conditioner" and tested after different periods. The conditioner was simply an arrangement by which air regulated as to temperature and humidity, was blown from the air supply over cotton-wool soaked in 10% peppermint and through a tube in which the insects were feeding on crushed wheat. The peppermint supply was renewed every 12 hr. Table 5B–E shows that of the animals tested after 8 and 27 days in the conditioner the percentage repelled by peppermint was not significantly greater than 50%, while of those tested after 3 and 16 days the percentage repelled was significant. Thorpe (1938) found similar fluctuations in his experiments on *Nemeritis*. The significance of the difference between the results of the control experiment and those given in Table 5E (27 days in conditioner) may be tested by calculating the χ^2 value, which proved equal to 17·12. For one degree of freedom, the chance of the two classifications being independent is less than 1 in 100, i.e. a real difference in behaviour towards peppermint has been brought about in *Rhizopertha* by keeping it for 27 days in a conditioner.

Table 5. *Production and extinction of conditioning effect* (*see Fig.* 1)

	No. in peppermint arm	No. in "blank" arm	Total	No. of experiments	No. of insects	S.E.	χ^2
Experiments after periods in "conditioner"							
A Not conditioned (control)	385	629 = 62%	1014	23	350	±1·57%	89·3
B 3 days	102	223 = 68·6%	325	10	100	±2·77%	22·7
C 8 ,,	120	150 = 55·6%	270	10	100	±3·04%	—
D 16 ,,	114	157 = 57·9%	271	10	100	±3·04%	14·3
E 27 ,,	184	181 = 49·7%	365	14	100	±2·62%	33·7
Experiments after periods in wheat:							
F 5 days	170	153 = 47·4%	323	20	150	±2·78%	—
G 10 ,,	160	202 = 55·8%	362	15	150	±2·63%	7·7
H 14 ,,	142	205 = 59·1%	347	20	150	±2·68%	13·4

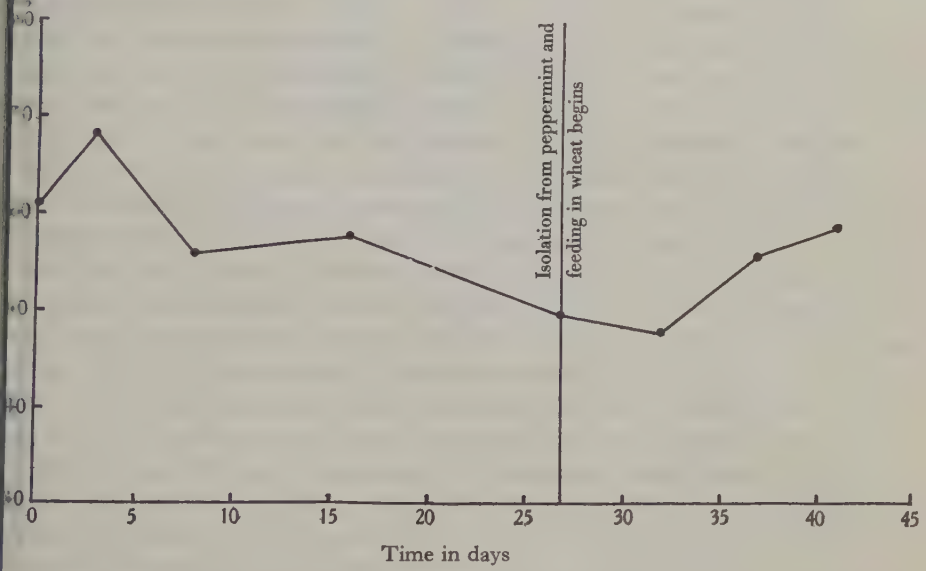
After 4 weeks in the conditioner, during which they acquired tolerance to the odour of peppermint, the insects were isolated from peppermint and fed on wheat and their responses to peppermint tested after different periods. Table 5F–H shows that after 14 days they are once again repelled by this odour. To test the significance of the difference between the results given in Table 5E (27 days in conditioner) and H (after 14 days' isolation from peppermint), the χ^2 value was calculated and

[1] The essence of peppermint used consisted of 10% by volume of "English white" oil of peppermint in 90% alcohol. The oil itself contained 46% free menthol and 9% as ester.

d equal to 5·09. For one degree of freedom this is significant, i.e. the con-ning effect has been really reduced. The production and extinction of the tioning effect are illustrated by Fig. 1.

VII. SUBSTANCES IN WHICH *RHIZOPERTHA* WAS REARED

ne hundred *Rhizopertha* were put into large Petri dishes each containing one umber of substances. The period of survival in each of these substances was ded. The animals lived from 8 to 15 days in salted peanut, ground soya beans, milk, cooked mustard, cooked green pea, vetch, rotten oak, and oak shavings; 20 to 44 days in Whatman's no. 1 filter paper, alfalfa, cork, cooked vetch,



1. Graph showing the production and extinction of the conditioning effect. ● Average propor-repelled by peppermint after various periods in conditioner, then various periods isolated from ermint in wheat (Table 5).

ed lentils, raw peanut, raw lentils and cooked haricot beans; from 100 to 146 s in whole and polished rice, tapioca, corn-flour, cooked maple pea and cooked d bean; and 179 days in arrowroot biscuit and rolled oats. After this period was still alive in raw haricot beans and five in peppermint sweets. Cultures e still alive after a year in wheat, barley, pearl barley, maize, oats, wheat with *ia Azedarach*, wholemeal flour, buckwheat and wheat which had been soaked o% peppermint solution.

VIII. DISCUSSION

The problem of "host selection" in *Rhizopertha dominica* presents a relatively ple case and the various steps leading up to the discovery of an environment situation suitable for oviposition lend themselves easily to examination by erimental methods. The animal appears to be first attracted to its food, in which

it also oviposits, by the sense of smell. Larvae and adults live on the same foo
Once within an environment smelling of food the sense of smell appears to lose i
importance and that of touch to become paramount: as many eggs are laid per da
on hemispheres of plain plaster as in those having the odour of food (§ IV). Th
animal is aware of the difference for after starving for 1 day it will give the feedin
response with objects having the odour of food and not with those of plain plast
in the same dish, but this awareness has no effect on its choice of oviposition sit
But to obtain full oviposition the animal must receive both olfactory and tacti
stimulation of an appropriate kind: otherwise restraint is exercised and eggs ar
laid later and in smaller numbers (Table 1). The olfactory receptors concerned ar
located in the antennae and legs, while tactile examinations are carried out mostl
by the palps. In its use of one sense to bring itself into a suitable environment an
another to locate the actual site for oviposition *Rhizopertha* bears some resemblanc
to *Alysia*, *Trichogramma* and other insects (Laing, 1937). *Trichogramma*, fo
instance, is first attracted by smell to traces left by *Sitotroga* females, then perceive
its hosts (eggs of *Sitotroga*) by sight, while *Alysia* is attracted by the smell of carrio
to an environment likely to contain its dipterous maggot hosts, but locates th
maggots by stabbing movements of the ovipositor. A slightly different case is tha
of the tsetse fly (*Glossina*) which hunts its host, cattle or men, mostly by sigh
(Bax, 1937), but once alighted on their skin the feeding response is elicited—excep
when very hungry—only by smell. Insects without antennae nearly always fail t
show this response (Nash, 1930). Also in *Mormoniella vitripennis*, Jacobi (193
found a succession of reactions depending on different senses. The host, the pup
of *Calliphora*, is found by smell, which calls forth the drumming reaction of th
antennae, and later, combined with some discrimination by the sense of touch
tapping with the abdomen, extrusion of the ovipositor and drilling. But no eg
are laid unless the ovipositor encounters an air space between the pupa and th
puparium, detected presumably by the sense of touch.

As the sense of smell is not used in the selection of oviposition site once th
animal is in an environment with the odour of food, it would be surprising if
exhibited any predilection, when offered the choice of objects containing variou
kinds of food, for laying eggs on the kind of food in which it had been reared; an
indeed it does not. Likewise it shows no greater attraction, in an olfactometer
towards the kind of food in which it was reared than towards other foods. Th
males and females have much the same intensity of attraction to food may sho
in the case of the females, as of course with the males, that it was an attraction f
feeding rather than for oviposition.

That *Rhizopertha* became tolerant to the odour of peppermint when kept
it under optimum conditions corroborates Thorpe's discoveries in *Nemeritis* an
Drosophila. As Thorpe (1938) has pointed out this is different from conditioni
in Pavlov's sense of the term. In Pavlov's (1927) experiments an association w
established between a neutral stimulus and a specific reaction formerly unconnect
with the stimulus. Thorpe showed that the type of response he obtained was
association of a given constituent of the environment (e.g. odour) with optimu
conditions of life as a whole, not with any single specialized response.

he conception of "gestalt" as developed by Köhler (1930) and others offers planation of such behaviour as that described above. This conception may pressed by saying that response in this case does not follow stimulus in itomatic manner of a reflex, but rather the constellation of stimuli providing ensation becomes organized in the brain or central nervous system, and nse is a result of the organized whole. The basis of the capacity to react to a n of excitation appears to be that the motor effects produced by the impulses ng in the central nervous system from any one receptor cell will differ according ether they arrive alone or accompanied by impulses from other receptors. viposition in *Rhizopertha*, for instance, does not take place unless the pattern citation has a certain character involving both olfactory and tactile sensation, he sensory pattern must be complete if restraint is to be avoided.

slightly different explanation is required to fit olfactory conditioning into the alt" system. The change in behaviour of *Rhizopertha* towards the odour of rmint, like that of *Nemeritis* towards cedar-wood oil, cannot be said to be more than "habituation" (see Humphrey, 1933). The response of the animal ds the odour becomes neutral instead of negative. On the other hand the nse of *Drosophila* to peppermint changed from negative to positive, as did also f the blowfly *Calliphora erythrocephala* towards menthol when it was exposed t odour during larval life (unpublished). These animals appear to be reacting a pattern but to this one sensation out of all those received by its sense organs. it appears that animals normally respond only to those sensations which are of icance. With *Bombyx mori*, for instance, males will attempt to mate with hed female scent glands, and ignore the otherwise intact female nearby (Kellogg, . Male *Limonius canus*, the adults of wireworms, will extend their genitalia try to mate with each other in the presence of certain fatty acids which rently have odours similar to the female's (Lehmann, 1932). The robin will ure at a detached red breast, but often gives no such response to an otherwise t stuffed specimen from which the breast has been removed (Lack, 1939). points at which an animal's mind, as seen in behaviour, makes contact with xternal world are few, but clearly adaptive: it does not respond to sensations h have no bearing on its own existence. The female scent and a red breast respectively a natural significance for *Bombyx* and the robin; but for *Calli-a* and *Drosophila* the odours of menthol and peppermint respectively have cially been made significant. This is effected by a process of conditioning eby the new characteristic of the situation, here the odour of peppermint, is introduced into the animal's perception or cognitive experience as a non-ficant sensation. Before a response can occur, therefore, it must be organized the existing significant sensations into a new pattern in the animal's brain. ther words, the animal would probably not respond to the new sensation ss it were introduced against a background of significant sensations, in this those resulting from optimum conditions of life, and the effect is that it takes ce in a particular way of a sensation it would otherwise have ignored, or to h it would at least have responded differently.

The problem of conditioning has a practical as well as a theoretical interest It may explain such large scale changes in habit as are found in many insects o economic importance, e.g. the Colorado beetle (*Leptinotarsa*), the parsley stal weevil (*Listronotus*), and, according to one theory, certain sheep blowflies i Australia (see Tillyard & Seddon, 1933). Also, as Imms (1931) has observed a knowledge of the sensory reactions of economic insects is often essential to devisin means of repression.

IX. SUMMARY

The senses used in food finding and the factors inducing oviposition in *Rhizo pertha dominica* (Fab.), a Bostrichid beetle pest of stored grain, were investigated The sense of smell appears to be the most important in leading the animal to a environment where food is present. Responses were obtained in an olfactomete with maize, wheat, oats and barley and with different extracts of wheat and maize The use of the sense of sight was also investigated.

Once within an environment having the odour of food the sense of touch appear to be paramount in the selection of oviposition site. The influence of size, shape an texture were investigated. But in the absence of appropriate patterns of stimul involving both olfactory and tactile sensations, restraint from oviposition wa exercised.

No predilection was shown for oviposition or feeding in the food in which the were reared as larvae. Neither did the larvae especially choose for entry substance in which their parents have been reared.

The animal can be made tolerant to the odour of peppermint instead of repelle by it in an olfactometer by exposing the adults to the odour of peppermint for a fe weeks. The effect wears off after 10–14 days' isolation from peppermint in wheat

Olfactory receptors which appear to be located on the antennae and legs wer used in both food-finding and recognition of the opposite sex.

A list is given of the substances in which the animal was reared.

The bearing of these results on the problem of "gestalt" in conditioning, an on changes in habit in economic insects, is discussed.

I wish to thank Dr W. H. Thorpe and Dr A. D. Imms, F.R.S., for their criticism and encouragement, Prof. James Gray, F.R.S., in whose laboratory the work wa done, Prof. Julius Seiler for accommodating me for a short time in his laborator in the Eidgenössische Technischen Hochschule, Zürich, and the various othe people who have given me advice.

REFERENCES

Abbott, C. E. (1927). *Ann. ent. Soc. Amer.* **20**, 207, 550.
Barnes, J. H. & Grove, A. J. (1916). *Mem. Dep. Agric. India, Chem.* **4**, 166.
Bax, S. N. (1937). *Bull. ent. Res.* **28**, 539.
Brecher, G. (1929). *Z. vergl. Physiol.* **10**, 495.
Chittenden, F. H. (1911). *Bull. U.S. Bur. Ent.* **96**, 29.

Craighead, F. C. (1921). *J. agric. Res.* **22**, 189.
—— (1923). *Canad. Ent.* **55**, 76.
Fisher, R. A. & Yates, F. (1938). *Statistical Tables.* London: Oliver and Boyd.
Hoskins, W. M. & Craig, R. (1934). *J. econ. Ent.* **27**, 1029.
Humphrey, G. (1933). *The Nature of Learning in its Relation to the Living System.* London: Kegan Paul, Trench, Trubner and Co.
Hunter, W. D. & Hinds, W. E. (1905). *U.S. Bur. Ent. Bull.* no. 51.
Imms, A. D. (1937). *Recent Advances in Entomology.* London: Methuen (1st edition 1931).
Jacobi, E. F. (1939). *Arch. Néerl. Zool.* **3**, 197.
Kellogg, V. L. (1907). *Biol. Bull. Wood's Hole*, **12**, 152.
Köhler (1930). *Gestalt Psychology.* London: J. Bell and Sons.
Lack, D. (1939). *Proc. zool. Soc. Lond.* A, **109**, 169.
Laing, J. (1937). *J. Anim. Ecol.* **6**, 298.
Larson, A. (1927). *Ann. ent. Soc. Amer.* **20**, 37.
Lehmann, R. S. (1932). *J. econ. Ent.* **25**, 949.
Marshall, J. (1935). *Trans. ent. Soc. Lond.* **83**, 49.
Minnich, D. E. (1922). *J. exp. Zool.* **36**, 445.
—— (1929). *Z. vergl. Physiol.* **11**, 1.
Nash, T. A. M. (1930). *Bull. ent. Res.* **21**, 201.
Pavlov, I. P. (1927). *Conditioned Reflexes.* London.
Picard (1922). *Bull. Biol.* **56**, 54.
Potter, C. (1935). *Trans. ent. Soc. Lond.* **83**, 449.
Purewal, S. S. & Rao, P. K. (1936). *Misc. Bull. Imp. Coun. agric. Res. Delhi*, no. 11, 1.
Richards, O. W. (1927). *Biol. Rev.* **2**, 298.
Salt, G. (1935). *Proc. Roy. Soc.* B, **117**, 413.
—— (1936). *Proc. Roy. Soc.* B, **122**, 57.
Thorpe, W. H. (1930). *Biol. Rev.* **5**, 177.
—— (1938). *Proc. Roy. Soc.* B, **126**, 370.
—— (1939*a*). *Proc. Roy. Soc.* B, **127**, 424.
—— (1939*b*). In *The New Systematics.* Edited by J. S. Huxley. Oxford.
Thorpe, W. H. & Jones, F. G. W. (1937). *Proc. Roy. Soc.* B, **124**, 56.
Tillyard, R. J. & Seddon, H. R. (1933). *Pamphl. Coun. sci. industr. Res., Aust.*, no. 37.
Ullyett, G. C. (1936). *Proc. Roy. Soc.* B, **120**, 253.
Uvarov, B. P. (1932). *V^e Congrès Internat. Ent.*, Paris.
Valentine, J. M. (1931). *J. exp. Zool.* **58**, 165.
Wigglesworth, V. B. & Gillett, J. D. (1934). *J. exp. Biol.* **11**, 120.

ON CHROMATIC EFFECTOR SPEED IN *XENOPUS* AND *ANGUILLA* AND THE LEVEL OF MELANOPHORE EXPANDING HORMONE IN EEL BLOOD

By H. WARING and F. W. LANDGREBE

Department of Natural History, University of Aberdeen

(*Received* 10 *November* 1940)

(With Nine Text-figures)

1. INTRODUCTION

Hogben (1924) first drew attention to a broad distinction between two classes chromatic behaviour. Where there is direct nervous control of the melanophor reversal from white to black backgrounds under overhead illumination and vi versa is rapid, and where co-ordination is by a blood-circulated hormone the chan is slow. Later work by this school has amply confirmed this generalization a emphasized the importance of accurate time analysis of normal responses to varyi visual stimuli before drawing far-reaching conclusions about the nature of t co-ordinating mechanism involved (Hogben & Slome, 1931, 1936; Smith, 193 Hogben & Landgrebe, 1940; Neill, 1940).

A correct interpretation of time relations requires data about the separate spee of transmission by the co-ordinating system and of effector reaction. We can assi limits to the speed of the nervous impulse in vertebrate animals, to transmissi across synapses in the central nervous system and to the excitatory processes with the receptor organs involved. The total interval occupied by all of these is small comparison with time intervals involved in overt chromatic behaviour.

The reaction time of the pigmentary effector organs of teleost species with ra chromatic responses has recently been determined by faradic stimulation and tre ment with drugs (Osterhage, 1932; Neill, 1940). The effector speed of such spec is comparatively slow and might well be a limiting factor in the time relations colour response. Hitherto no similar analysis has been based on animals with sl response and humoral co-ordination. Fenn's (1924) experiments suggest that t melanophore speed of *Rana* is about 30 min., but they were based on the macrosco change in a perfused limb. It has been repeatedly demonstrated in recent ye that such criteria are useless for exact comparison. Young (1935) recorded graphica the contraction of melanophores in isolated lamprey skin immersed in physiologi saline. His data, compiled for another purpose, are not complete but show t the melanophore speed is very slow.

The following experiments were made to find the chromatic effector speed two species for which exhaustive analysis of the normal responses has been ma

lly the eel and the South African clawed toad. The same apparatus was used oth. It consisted of two reservoirs, one for saline and the other for saline with l pituitary extract or adrenalin, both connected by a two-way tap with a constant ure cannula. The following saline solution (buffered to *p*H 7·5 with Na phos-) was found suitable for both: 0·15*M* NaCl (buffered), 100 c.c.; 0·15*M* KCl, c.c.; 0·15*M* $CaCl_26H_2O$, 1·75 c.c.; 0·15*M* $MgCl_26H_2O$, 1·5 c.c. The various ions were kept separate and mixed just prior to each experiment. Pressor free sterior lobe pituitary extract was prepared as prescribed by Hogben & Gordon). The pituitary lobes were separated within 20 min. of the animals being and plunged into acetone. When sufficient were obtained a stock solution ining 5 % fresh (wet weight) substance was prepared, boiled and sealed in tubes. Experimental procedure was similar for both species. The hearts of animals ibrated on an illuminated black background (melanophores expanded) were ulated via the sinus venosus and the blood was washed out. Frequent readings e melanophore index (μ) were taken in accordance with the Hogben scale ben & Slome, 1931). Readings were taken on the posterior web of *Xenopus* n the pectoral fin of *Anguilla*.

The data obtained are shown graphically. When the melanophores had reached ibrium at the lower reading (e.g. $\mu = circa$ 1·5) a further 20 min. perfusion saline was continued, and only then, by turning the two-way tap, was saline aining pituitary extract brought into circulation.

The lowest value for μ given by the eel after perfusion with saline is 1·5. As d be expected from other data (Waring, 1940) this mean figure for an area of ectoral fin is lower than the average index from the whole animal. Separate ings from four areas on the tail of hypophysectomized animals gave a mean μ ·7–3·2, according to lighting conditions. On the pectoral fin it is possible to t for observation areas in which μ reaches 1·5. In the graphs correction has made for the time taken for the new solution to reach the area of observation. s correction was based on observations made using innocuous dyes.

2. EFFECTOR REACTION TIME TO PITUITARY EXTRACTS

The minimum concentration of pituitary extract necessary to bring about ansion was first determined. Thereafter various concentrations were tested to whether the speed of effector response varies significantly with concentration. following concentrations all evoked maximal responses, with the exception of first shown:

Xenopus	Eel
0·0025 %	0·00015 %
0·005	0·000625
0·0125	0·00125
0·025	0·0025
0·025	0·005
0·0375	0·01
0·05	0·015
—	0·025

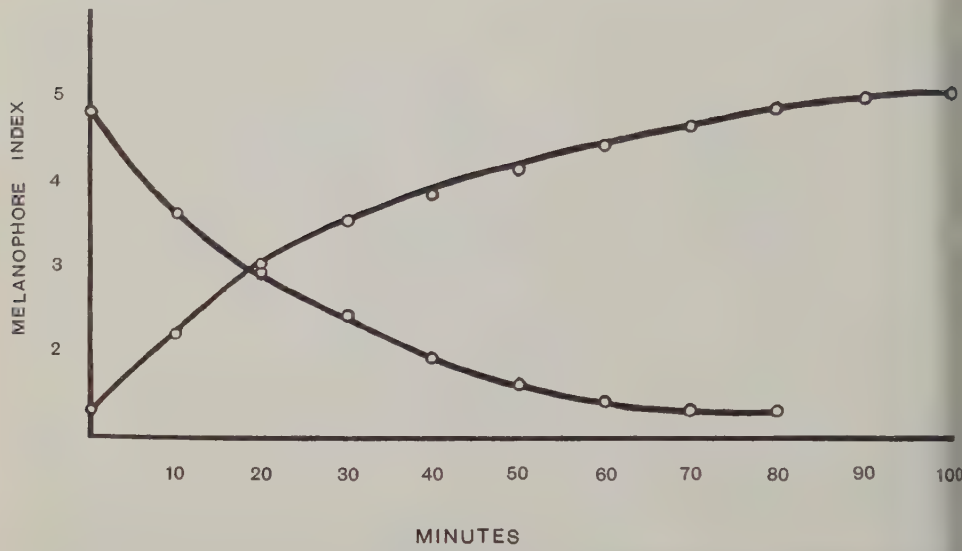


Fig. 1. *Xenopus*. The speed of melanophore contraction and expansion in preparations perfused with saline and saline containing pituitary extract. Each point is the average reading from six preparations. Temp. 16–18° C.

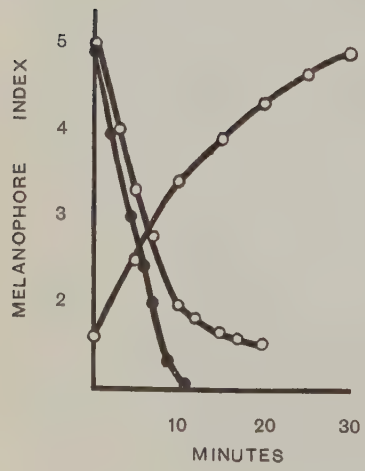


Fig. 2.

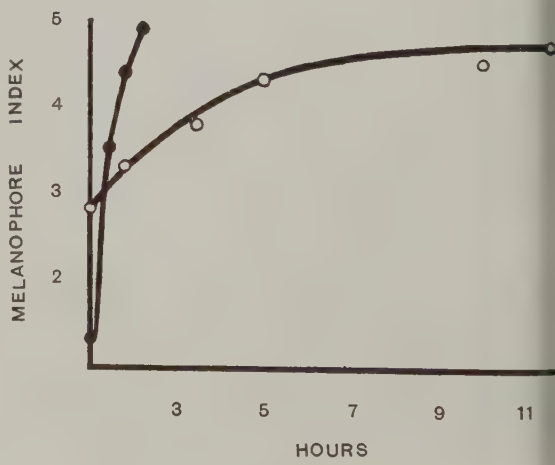


Fig. 3.

Fig. 2. *Eel*. The speed of melanophore contraction and expansion in preparations perfused with saline and saline containing pituitary extract. Each point is the average reading from seven preparations. Temp. 17–20° C. –●—●– Response to adrenalin.

Fig. 3. *Xenopus*. Comparison of the melanophore response of normal animals brought into black illuminated tank after 38 days in total darkness (22° C.) and the melanophore response in preparations perfused with saline containing pituitary extract. Temp. 16–18° C. –○—○– Normal animals (from Neill, unpublished). –●—●– Perfused preparations.

he speed of reaction did not vary significantly in response to any concentrations
n were found to evoke full response, and the graphs of Figs. 1 and 2 are there-
representative of the course of events in all experiments with concentrations
0·0025% (*Xenopus*) or 0·00015% (*Anguilla*). At the subthreshold dose
25%) melanophores of *Xenopus* uniformly expand to approximately $\mu = 2$ and
ain this condition. Subthreshold doses with the eel evoke a different and
ilar response. Some of the melanophores expand slightly and others undergo
xpansion in the normal time.

he two most striking facts which emerge from these data are the greater
ivity of the eel melanophores to pituitrin and the greater speed of their
nse. The melanophore speed of *Xenopus* is very slow; but even so it is not the
ng factor in the slow normal reaction. This is clearly shown (Fig. 3) by plotting
elanophore response to perfusion on the same graph as that for the build-up
e melanophore expanding hormone (B hormone, Hogben & Slome, 1931) in
ormal animal following transition from complete darkness to an illuminated
background. The responses plotted in Fig. 1 also show that the super- and
ormal curves recorded from observations on intact animals in transition from
ess to illuminated black backgrounds and the reverse (Hogben & Slome, 1931)
t, as the authors infer, from changes in the co-ordinating system and are not
o any physical peculiarity of the melanophore.

3. THE ABSOLUTE QUANTITY OF B IN THE BLOOD OF AN EEL

n the basis of these results it is now possible to make direct determinations
e level of the B hormone in eel blood. Preliminary attempts to inject eel serum
hypophysectomized *Xenopus* were not satisfactory. Although the presence of
elanophore substance was detectable, pathological symptons regularly super-
d.

he method finally adopted was to decapitate eels with expanded melanophores
der to ensure that the pituitary itself did not contaminate the serum, and drain
lood, as in previous work (Waring, 1938). This precaution is necessary because
e high level of B hormone in the pituitary gland of various vertebrates (Hogben,
; Waring, 1936*a*, *b*). Clear serum was prepared by centrifuging the fresh
d and injected direct into groups of hypophysectomized eels of equal weight.
ophysectomized recipients were used because recent work in other fields has
n that pituitary injections may call forth endogenous secretion of pituitary
ones. The melanophore response to the injected blood was then plotted and
pared with the response to various injections of B containing extract dissolved
e same bulk of eel Ringer (p. 81). The responses showed consistent dis-
ination between various doses (Fig. 4). The response to a blood injection
donor fish with an average μ of 4·5 is not given: it did not differ significantly
response to 0·005% pituitary extract.

he pituitary extract used for assay was from the *same sample* as that used in
erfusion experiments. Prima facie we may thus draw the following conclusions
these experiments:

(*a*) Since 3 c.c. of blood evoke the same response as 3 c.c. of 0·005–0·01 pituitary (B) perfusate, the blood contained the B equivalent of 0·005–0·01 pituitary extract.

(*b*) Since the minimum concentration necessary to evoke expansion (p. 81) 0·00015–0·0003 % pituitary extract, blood of dark eels contains at least 15 tim the threshold concentration.

In this connexion it must be noted that the highest average reading of the don fish was 4·8. Given time and appropriate lighting conditions eels equilibrate on illuminated black background at 5·0 (Neill, 1940) and there is therefore no reas to believe that the figure given represents the full maximum of circulating B hormo that may be present in eels.

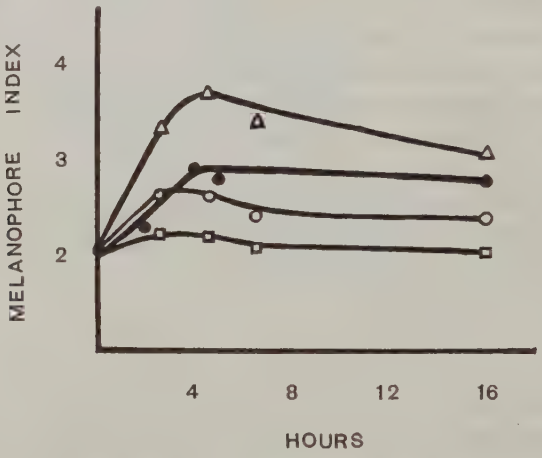


Fig. 4. Responses of hypophysectomized eels to various doses of pressor free posterior lobe extr and serum removed from black adapted eels. Each point is the average reading from the same f eels of equal weight. –●—●– 3 c.c. serum from donor fish $\mu = 4{\cdot}8$. –△—△–3 c.c. of 0·01 % pituit extract (i.e. 0·2 c.c. 5 % pituitary extract in 100 c.c. saline). –○—○– 3 c.c. of 0·005 % pituit extract. –□—□– 3 c.c. of 0·0025 % pituitary extract.

Some reference is necessary to the nature of the standard extract used. Ma malian posterior lobe extracts have at least four properties: oxytocic, renal, pres and melanophore. Eel extracts exhibit the last two; the two former have not be tested for since (*a*) oxytocin has no general effect on plain muscle (blood vesse or melanophores, (*b*) if diuresis inhibiting power has any part in the melanoph response to B, it is overruled by pressor activity which is here antagonistic to (p. 95). As judged by the melanophore index, injections of pressor contain posterior lobe are significantly less potent than are caustic soda treated pres free extracts. A likely interpretation of this is that pressor substance, by its va constrictor action, limits the availability of B to the tissues (Hogben & Gord 1930). If this is true it follows also that the ratio of pressor to B substance in blood is such that in injected extracts the former has a significant effect on distribution of the latter. Whether these autacoids are in similar proportions in circulatory system is unknown. Thus we do not know whether blood samples w

rable quantities of B have necessarily significant quantities of pressor sub-. Both Krogh and Hogben have shown that the amount of pressor hormone ating in the frog's blood is of physiological significance in maintaining the dar tone of the vessels. If 3 c.c. of blood from the donor fish used in the nt experiments contained significant quantities of pressor substance the estimate B content is still valid for the effective level of B but less than the *absolute* ity.

4. TOTAL BLOOD VOLUME OF THE EEL

a later section data are given of the amount of B hormone present in the ary glands of eels (71 g.) kept in a black tank with overhead illumination. When tal blood volume of fish of similar size is known we can then say approxi- how much B hormone is in circulation relative to the amount in the gland. hen blood is drained from eels for blood transfusion experiments the yield ndividuals of the same size and weight varies considerably. A similar variation ound when attempts were made to measure the total blood volume. The ple used was to compare colorimetrically the haemoglobin content of the rized whole carcass with that of a known volume of blood previously withdrawn the same animal. The eel has "white" flesh. It was assumed, therefore, that tra vascular haemoglobin included would fall within the limits of experimental

fter numerous preliminary experiments the following procedure was finally ed:

) Eels of approximately the required size were selected, weighed and their cement of water measured. The displacement is easy to measure if a measuring der of the correct diameter is used and the live eel is inserted head first. The wims straight to the bottom and is allowed just sufficient space to turn on so that the head eventually comes to the surface. The duration of complete ergence is ample to provide a reading.

) The animals were injected intravenously with 1 c.c. of 10% sodium citrate pproximately 1 hr. after injection 0·1 c.c. of blood was pipetted from the heart ransferred to a 10 c.c. measuring flask containing approximately 9 c.c. of water sufficient citrate to make the final 10 c.c. a 0·1% citrate solution. This con- ation of citrate is well below an amount usually considered necessary to inhibit olysis. The blood dilution usually recommended for complete haemolysis is oo but 1 in 100 was found to be the maximum dilution consistent with accurate r comparison.

) The large gall bladder was punctured and dried out with a cotton swab and whole body cut into small pieces and immersed in water containing sufficient e to make the final yield (below) 0·1% citrate. The fluid was pressed out of arcass and collected. The carcass was then pulverized and extracted with ilar citrate solution. This second yield, unlike the first, was contaminated with particles which could not be removed with any of the solvents tried. It was fore subjected to repeated filtration at low temperature and the filtrate was

added to the first yield. Correction for the amount of fluid taken up by the carca and not expelled by pressing was obtained by measuring the displacement of t pulverized carcass and comparing it with the volume obtained for the whole anim The final yield was made up to correspond approximately with the dilution us with the 0·1 c.c. blood sample first taken, e.g. to 100 c.c. for animals which experien showed were likely to yield 1 c.c. of blood.

Finally, HCl was added to both the carcass washings and the first sample. slightly stronger than $N/100$ the solutions turn from red to brown. The end poi is fairly sharp. Further small additions of HCl do not change the colour. Aft filtration the two solutions were matched against each other and after adjustme by dilution the total blood volume of the animal was calculated.

The results obtained cannot be taken as exactly indicating the blood volum but do represent an approximation sufficient for the present purpose:

No.	Weight g.	Length in.	Displacement c.c.	Blood volume c.c.
1	40	—	42	1·25
2	47	14½	45	1·4
3	48	13½	50	1·0
4	51	—	48	1·0
5	56	14	50	2·5
6	78	15½	70	2·0
7	82	16	75	2·5
8	82	16¼	75	2·8
9	92	16	80	3·0
10	98	—	96	1·9
11	103	16½	100	2·0

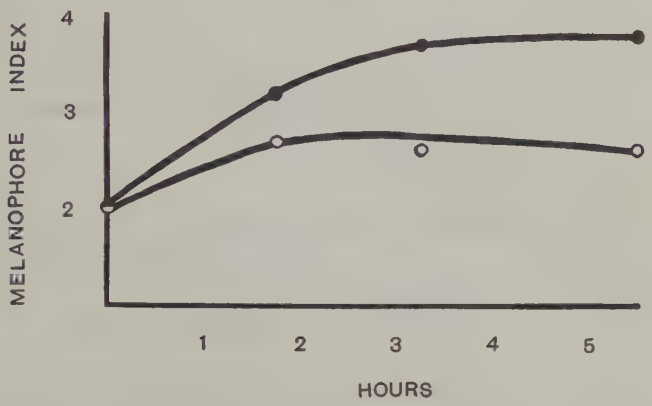
5. THE QUANTITY OF B HORMONE IN THE PITUITARY OF THE EE

Estimations of the quantity of melanophore hormone in the pituitary of varic species have been made. Hogben (1924) found that there was sufficient substan in one frog gland to darken fifty-six pale frogs, Lundstrom & Bard (1932) extract sufficient from one *Mustelus* gland to darken four pale hypophysectomized fi and Waring (1936*a*) obtained similar results from another dogfish (*Scyllium*). The estimations were made on fresh extracts but the lighting conditions to which t donor animals were subjected were not specified.

In the present investigation more exact estimates were made possible by the u of hypophysectomized *Xenopus*. *Xenopus* has several advantages over other spec as a test animal. It lives indefinitely in captivity and is unaffected by repeat handling and injection, so that estimations can be repeatedly checked against standa pituitary extracts. This advantage is not shared by any other animal in our experien The importance of a reliable test animal would hardly seem to merit comment, b for the fact that many investigators have drawn conclusions from experiments usi small numbers of frogs as test animals. Frogs (*Rana temporaria*) are not satisfact test animals for several reasons. Different individuals vary enormously in th response to humidity. The temperature of a subaquatic medium is not so easy stabilize as water. Until recently we have not been able to maintain frogs un

tory conditions for sufficiently long periods, and even with the methods now he time taken for routine maintenance is a serious disadvantage.

ne use of hypophysectomized *Xenopus* is to be preferred to intact ones for llowing reasons. They are much more sensitive to B. Within a wide range re not susceptible to changes of illumination. Their use forestalls any objection he responses obtained are wholly or partly due to the endogenous secretion uitary autacoids under the influence of the injected substance. Only one s source of error must be guarded against in the use of these animals. Their ivity increases slowly for a number of weeks after operation, after which they mpletely reliable. Detailed data on their behaviour will shortly be published this laboratory.



Response of *Xenopus* to equal doses of the same posterior lobe extract. Cold *N* caustic soda n added to both doses. –○—○– Neutralized immediately with HCl. –●—●– Neutralized ater with HCl. Drawn from data given in Hogben & Gordon (1930).

he pituitaries were dissected from seven eels of average weight, 71 g., which previously been maintained in a black tank with overhead illumination for nths. A sterile aqueous extract was prepared, 2 c.c. of which was equivalent e pituitary. This extract was boiled and sealed in tubes but was not treated caustic soda. Hogben & Gordon (1930) showed that when a posterior lobe ary extract is treated with normal caustic soda in the cold at least nine-tenths pressor activity is destroyed and there is a marked increase in its melanophore nding potency. Part of their data is replotted in Fig. 5. They attributed this ase of potency to the greater availability of B to the tissues in the absence of ressor component. Since the present aim was to obtain data on the available B one in the pituitary on the assumption that the blood would take up both B asopressin in the intact animal, extracts were not treated with caustic soda. ne other hand, comparison was required with the standard pressor free extract n was used for determining the threshold dose of the melanophores (p. 81) f the percentage of effective B in circulation in the fully dark animal (p. 84). ig. 6 shows the response of the test animals to injections of eel pituitary ct and of the standard pressor free extract. Certain aspects of these curves

are discussed in detail later in this paper (p. 90). Meanwhile the data recorde show (in terms of melanophore expanding power):

1 c.c. of extract containing half an eel pituitary ≡ 1 c.c. of standard 0·0006 % ox pituitary extract (p. 81).

∴ 1 eel pituitary ≡ 1 c.c. of 0·0012 % of standard ox pituitary extract, i.e. 1 e*pituitary contains the B equivalent of* 0·000012 *g.* (*wet weight*) *of ox posterior lob pituitary.*

From data previously given (p. 84) we know that:

Eel blood ($\mu = 4{\cdot}8$) contains the equivalent of 0·005–0·01 % standard ox pituitar extract, i.e. 100 c.c. eel blood are equivalent to 0·005–0·01 g. ox posterior lob pituitary.

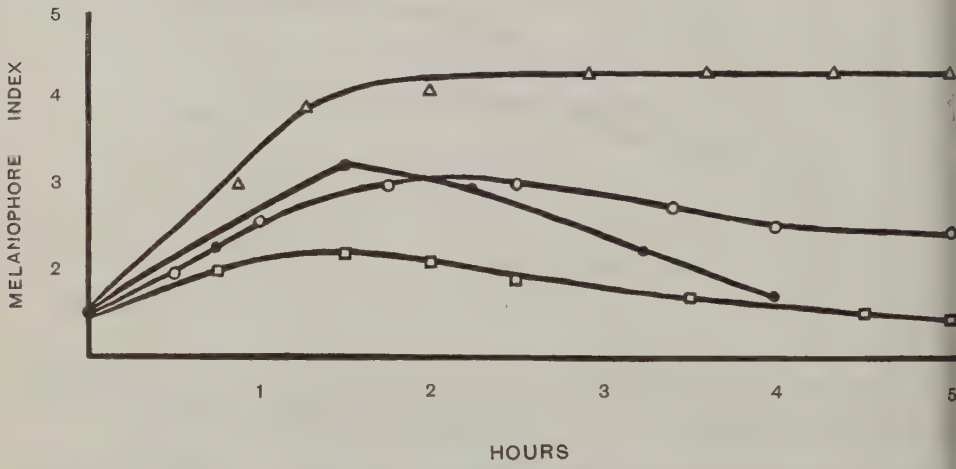


Fig. 6. Responses of same group of hypophysectomized *Xenopus* to various extracts (see text). A injections 1 c.c. white tin. Illuminated. 15° C. –●—●– Extract equivalent to half an eel pituitar Not treated with caustic soda. –△—△–0·0025 % ox pituitary extract treated with cold caus soda. –○—○– 0·0006 % ox pituitary extract treated with cold caustic soda. –□—□– 0·0003 % pituitary extract treated with cold caustic soda.

From the data on p. 86 we know that a 70 g. eel contains approximately 2 c blood. So that the circulation of 70 g. eels contain the B equivalent of 0·000 0·0002 g. of ox posterior pituitary.

We may, therefore, say that the relative amounts of B substance in the pituitary a in the circulation of illuminated dark eels is of the order: pituitary 1, circulation 1

These values can only be approximate because of two sources of error whi we cannot allow for at present. One is seasonal variation in the potency of glandul extracts and possibly a similar variation in recipient sensitivity, the other is a speci specificity in response to hormone injections. Waring (1936*b*) described a seasor variation in the B content of the frog's pituitary. His data were based on i sufficient numbers for detailed conclusions and the work is now being repeate but one fact emerged quite clearly. During March there is in *Rana* a *sharp* decli in the melanophore potency of pituitary extracts. Waring found no evidence (usi

tract as a standard) for a comparable variation in recipient sensitivity. Jores , however, claims that the latter exists. The existence of such variables means omparative estimations of B content of serum and pituitary are suspect in nimal unless they are carried out absolutely simultaneously—which was not le in this work—or the absence of a seasonal variation has been proved. We not examined eel pituitaries for such a variation.

evious work (Waring unpublished) on the relative potency of frog and dogfish ary extracts judged by reciprocal injection into frogs and dogfish, using ox ior lobe extract as a standard, indicated some measure of species specificity. er words frog pituitary extract was more potent when judged by the response g melanophores, than when judged by the response of dogfish melanophores ice versa. Since then Creaser & Gorbmann (1939) have collected extensive rom various sources dealing with a variety of species which shows that there ry definite species specificity of pituitary gonadotropins. In this investigation content of the eel pituitaries was estimated on *Xenopus* because this method fes the most accurate assay (p. 87). A parallel series of experiments was using hypophysectomized eels as test animals. Such estimations are laborious. elimination (or destruction) of B is very slow in hypophysectomized eels ng, 1940) and this together with the low viability of frequently handled eels it difficult to complete conclusive tests using the same group of animals. his reason the data are less complete than those cited from *Xenopus*. The d data available, however, indicate that the potency of eel pituitary extracts surably greater (*circa* 4 times) when estimated on eels as compared with ations using *Xenopus* as the recipient.

6. EFFECTOR REACTION TIME OF MELANOPHORES IN RESPONSE TO ADRENALIN

drenalin (0·001 % in saline) causes a rapid concentration of the pigment in elanophores of *Anguilla* even after prolonged perfusion with saline containing cess of B. The response shown in Fig. 2 was obtained immediately following in. perfusion with saline containing 0·5 c.c. stock pituitary per 100 c.c. In ast the drug gives no significant difference from straight saline when perfused gh a normal dark *Xenopus*. The strength of adrenalin which the heart of *pus* will tolerate is 8 times smaller than that used for eels.

nvestigation of numerous species of teleosts and reptiles having direct nervous ol of the melanophores has shown that adrenalin causes rapid concentration gment (see Summaries in Hogben, 1924; Sand, 1935; Parker, 1936, 1938).

oung (1935) showed that the melanophores of isolated skin fragments of *petra* immersed in saline containing adrenalin contracted more rapidly than immersed in physiological saline alone. Kleinholz (1938) found that intra-oneal injections of adrenalin evoked pallor in *Anolis*. In neither of these forms ne melanophores under direct nervous control.

xperiments with amphibians have not yielded uniform results. Lieben (1906), er & Scatterty (1937) and others have recorded concentration of pigment after

injection of adrenalin. Intraperitoneal injections of adrenalin into dark *Xenopus* evok no chromatic response. The clue to the discrepancy is probably to be found i Hogben & Winton's (1923) finding that intravenous but not intraperitoneal injectio of adrenalin into frogs causes pallor, a result which is not surprising in view of th ease with which adrenalin is oxidized. The drug has a marked vaso-constricto action and in amphibia probably brings about concentration of pigment by deprivin the melanophore of B circulated hormone. This conclusion is consistent with th finding here recorded that when the whole *Xenopus* preparation is washed free blood pigment concentration is no more rapid if the saline perfusate contai adrenalin.

The vaso-constrictor action of adrenalin probably plays a large part in melanophore contracting powers in all forms but we suspect that in the eel an probably other forms with direct nervous control it also has a direct effect on th melanophores (or on the nerves supplying them) for two reasons: (*a*) the respon is extremely rapid, (*b*) perfusion with straight saline after perfusion with sali containing B does not lead to the contraction of melanophores, but saline containi adrenalin evokes the rapid contraction already described.

7. MELANOPHORE RESPONSE TO VARIOUSLY TREATED B CONTAINING EXTRACTS

Fig. 6 shows that injections of eel and ox pituitary extracts which evoke a simil rise in index, do not maintain the higher index for similar periods. The more rap decline in the former may be due to some specific property of the eel extract or a change in the properties of a pituitary extract after treatment with caust soda.

The first possibility was eliminated by injecting eel pituitary extracts after trea ment with caustic soda, and ox posterior lobe without treatment, in doses whi evoked a similar rise in index (Fig. 7). A similar rise was obtained by trial wi a variety of doses. Only the relevant ones are recorded. The more rapid decli after injection of untreated extracts is greatly emphasized by using a larger do (Fig. 8).

Caustic soda treatment of general posterior lobe extracts has two well-defin effects on their melanophoric properties:

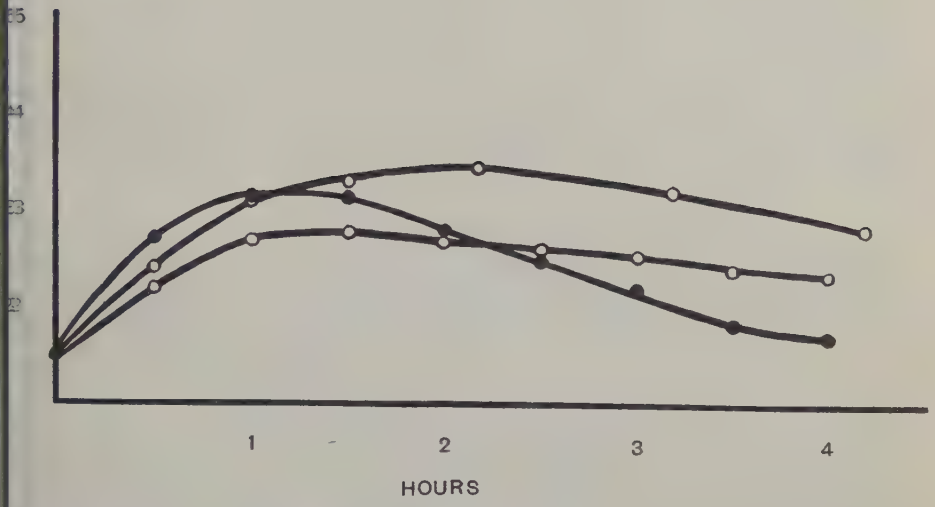
(*a*) There is a considerable increase in their potency in raising the melanopho index.[1]

(*b*) Doses of treated and untreated extract which raise the melanophore ind to the same level do not maintain the animal at this level for the same period of tim

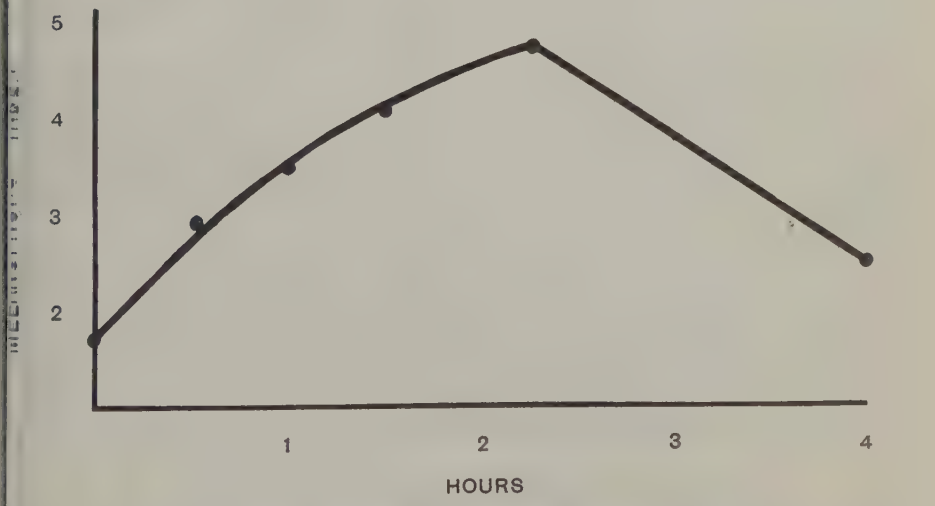
Caustic soda treatment of pituitary extracts may affect their melanopho properties in at least four ways:

(*a*) By acting on some constituent of the extract with no melanophoric properti to form B, so that there is an absolute increase of B.

[1] Jores (1933) claimed a 200–300% increase. Stehle (1936) considered Jores's estimate c servative. Abramowitz (1937) recorded an increase of 25 times using *Fundulus* pituitaries.



Responses of same group of *Xenopus* as Fig. 5 under same conditions. All injections 1 c.c. –○– Eel pituitary extract treated with caustic soda. –●—●– Ox pituitary extract not treated caustic soda. Ox pituitary extract contained $\frac{1}{250}$ of an international pressor unit.



Response of same group of *Xenopus* as Fig. 6 under same conditions to injection of ox posterior lobe containing $\frac{1}{60}$ of an international pressor unit.

(*b*) By modification of the extract so that the hormone is less easily excret or destroyed.

(*c*) By destruction of the pressor autacoid so that the availability of B to t tissues is increased.

(*d*) By modification of the B molecule itself so that (1) the potency of the extra is increased, and (2) the rate of excretion or destruction of the autacoid is reduce

It is convenient to consider these separately.

(*a*) *Absolute increase in B*

The view that caustic soda treatment increases the melanophore potency pituitary extracts by causing an absolute increase of B is based chiefly on exper ments with pituitaries removed from animals in darkness. Evidence of differe workers is contradictory. Koller & Rodewald (1933) claimed that extracts pituitaries from frogs maintained in complete darkness have a lower melanopho expanding potency than similar extracts prepared from frogs maintained und constant illumination. Jores (Jores, 1934; Jores & Will, 1934; Jores & Hoeltz, 193 confirmed this result using both saline and weak acid for extraction, but claim that the difference between the two extracts was eliminated by the use of caus soda extraction. He interpreted this in the following way. In darkness the precurs to B is present in the pituitary but very little effective B. Caustic soda acts on t precursor to produce definitive B. In a more recent paper Rodewald (1935) h been unable to confirm Jores' findings.

Kleinholz & Rahn (1940) found no significant difference between caustic so extracts of pituitaries from frogs maintained in darkness and under consta illumination. They did not make comparable extracts using neutral saline or we acid. Masselin (1939) found that pituitaries from *Bufo* kept in darkness for 14 da show an increase in B content but that shorter periods in darkness were witho effect. His experiments with different methods of extraction led him to conclu that alkaline, acid or saline extracts of pituitaries from animals kept under li conditions were equal in their B content.

In the face of such conflicting testimony it is clearly impossible to draw a conclusion. It may be noted, however, that even if there is a B-precursor in t gland under certain circumstances which can be activated by caustic soda, this c only explain the increased expanding power and not the flattened time-effect cur To explain the latter some other effect of the treatment must be invoked, e modification of the rate at which the substance injected is excreted or destroyed

(*b*) *Modification of excretion and/or destruction rate of B*

Whether B is ultimately excreted or is destroyed by the tissues is not know What little evidence exists points to the latter (Landgrebe & Waring, unpublishe If, however, B is passed by the kidney, the presence of renal regulators in the inject fluid would be expected to have an important effect on the shape of the respo curve.

e confusion which has hitherto characterized the literature on the relation itary autacoids to diuresis and its inhibition has recently been clarified by 's papers (1939–40) on mammals. The diuresis[1] which follows posterior ry injection into anaesthetized mammals is evoked by the pressor autacoid. ntidiuretic[1] effect in unanaesthetized animals is due to a substance present pressin fractions but separable from pressor by its heat resistance. If renal y enters into these considerations at all, we should expect that antidiuretic y would tend to prolong responses. Using Burn's (1931) apparatus for ing urine we injected standard caustic treated extract and untreated extract ice. The latter showed a well-marked inhibition of diuresis, the former no change that we could measure. So if B is excreted by the kidney, treat- of the extract with caustic soda would have the opposite effect on the time curve to that we obtained.

(*c*) *Destruction of the pressor component*

fore drawing any conclusions under this heading it was considered advisable the effect of untreated eel pituitary extract on blood pressure. Fifty eel ries were dissected and immediately dropped into 100 c.c. of pure acetone. gland was placed in the acetone within 3 min. of the donor being killed. The e was changed 24 hr. later and the glands stored in this fluid. Extracts were ed by boiling the dried pulverized material for 10 min. with mammalian Pressor assays were made on the anaesthetized cat (dial) with severed vagi. our was allowed to elapse between each test as recommended by Hogben *et al.* .

jection of 1 c.c. of extract equivalent to 5 mg. of dried eel pituitary evoked ressor response followed by a slight rise in blood pressure. Hogben (1927) scussed fully the phenomenon of depressor responses to pituitary injection. thetized cats with the vagi cut show a depressor response to extracts containing nine. Prolonged extraction of pituitary substance with ethyl alcohol removes epressant action. It would be surprising if our freshly desiccated substance ned appreciable quantities of histamine, but it was considered advisable to ae effect of extracting with alcohol to remove any histamine or histamine-like nce that might be present. The dried eel pituitaries were immersed in a few f absolute alcohol for 6 hr., dried, pulverized and extracted as before. The alent of 5 mg. now evoked a rise in blood pressure which was matched by an ion of 0·2 international pressor units. There was still a slight depressant action to the rise which could possibly be eliminated by more prolonged alcohol tion.[2] Shortage of material prevented us from making this test. It would appear that our extracts contained histamine or a histamine-like substance. is little reason to suppose that the depressant action was due to the presence anterior lobe in our material.

[1] As measured by urine volume.
[2] Pressor itself is slightly soluble in ethyl alcohol.

The only other teleost pituitary material that has been used for pressor assa to our knowledge is the cod (Hogben & de Beer, 1925). 10 mg. of cod pituita evoked a decided rise of blood pressure in the decerebrate cat. Extracts containin histamine have no depressant action on the blood pressure of such preparations s that it is not possible to say whether fresh cod pituitary contains a similar alcoh soluble substance to that found in the eel.

With the data available it is not possible to assess accurately the relative press potencies of eel and cod pituitary.

Should the amount of pressor substance in the dilutions used for melanopho assay (Figs. 7, 8) appear insufficient to have a significant effect on the peripher circulation, the following citation from Hogben (1927) confirms that extreme small doses evoke constriction. "Krogh has shown that visible effects on the capi

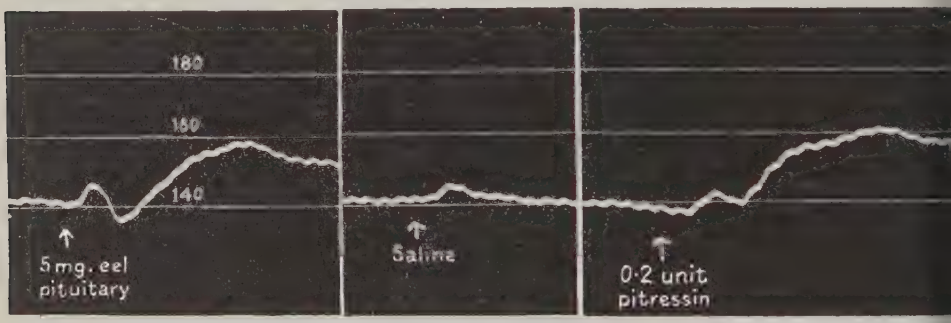


Fig. 9. Anaesthetized cat (dial). Both vagi severed. Carotid blood pressure. All injections i c
A, 5 mg. of desiccated eel pituitary; B, saline; C, 0·2 international pressor units.

laries alone are produced when the vessels are perfused with a Ringer containi a concentration of one in a million commercial pituitary preparation. Assumi the latter to be a 10% extract, this represents a dilution of 1 : 10,000,000 fre glandular substance!"

These considerations show that the quantities of pressor in the injected flu (Figs. 7, 8) were probably ample to have a marked effect on the peripheral circu tion. Whether it is shown by subsequent investigation that the effects caused caustic treatment are chiefly due to destruction of the pressor component as su or to some other change in the extract, two things need emphasis:

(*a*) Pressor in B extracts does have a very marked effect on the distribution o as judged by reading the web of *Xenopus* at different locations. Also large injectio of pitressin evoke a macroscopically apparent patchwork of light and dark ar in *Scyllium* (Waring, unpublished).

(*b*) Comparison of Fig. 7 with Fig. 8 shows that an increase of pressor togeth with a comparable increase of B, leads to a very sharp fall in the melanophore ind once the response has passed its peak.

is, therefore, difficult to avoid the conclusion that the effects of caustic ment are due in some measure to the destruction of the pressor substance h.

(*d*) *Modification of the B molecule*

res *et al.* (Jores & Lenssen, 1933; Jores & Will, 1934), Böttger (1937) and (1936, 1938) concluded that caustic soda treatment modified the B molecule Both the latter authors noted the longer duration of the effect after caustic ment. Stehle clearly envisaged the problem in the following way. The two observed after treatment may be due to destruction of the pressor autacoid modification of the B molecule. He accepted the second interpretation since c treatment of his own B preparation (which has a very low pressor content) rought about the characteristic effects.

e validity of this interpretation is questionable in view of work at present in ss by the present authors on a new B preparation with no measurable pressor rties in doses up to 1000 melanophore units. Caustic soda treatment of this nce has no effect on the response it evokes. Lest this be taken to imply that fects of caustic soda treatment are merely to destroy the pressor autacoid, even as in the case of Stehle's extract, the latter is present in only small quantities, be said that this simple interpretation is not feasible. The preparation referred okes a response similar to that of an untreated B extract containing pressor nlike that of a pressor containing extract after treatment with caustic. There veral possible interpretations of this. In the present context, however, the cited support the belief that Stehle's evidence, as it stands, is insufficient to nt the conclusion that the B molecule itself can be modified by caustic soda ment.

CONCLUSIONS AND SUMMARY

gben and his co-workers (Hogben & Slome, 1931, 1936; Neill, 1940) have a conclusions from the time relations of the responses of intact *Xenopus* and hich presuppose that the limiting factor in the slow responses is the equilibra- f the co-ordinating pituitary hormones in the blood and is not due to the a of the melanophores. The present investigation has shown that this sup- on is justified.

The chromatic effector speed of the two species has been measured by per- fully dark animals with saline until they were fully pale and then introducing mone into the perfusate to observe the reverse change. It was found that:

) Complete expansion of the melanophores of *Xenopus* takes 100 min. Com- contraction of the melanophores of *Xenopus* takes 70 min.

) Complete expansion of the melanophores of the eel takes 30 min. Complete action of the melanophores of the eel takes 20 min.

drenalin exercises a potent contracting effect on the melanophores of the eel in the presence of excess B. It has no direct effect on the melanophores of *pus*.

2. Increased concentration of B hormone in the perfusate above the thresho dose does not increase the speed of melanophore expansion in either animal. Th implies something approaching an all or nothing reaction on the part of the melan phore itself. The usual interpretation of the "all or nothing" behaviour of effecto is that it is in large measure due to the well-known "all or nothing" action of the efferent nerves.

3. Using eels of approximately the same weight estimations of:

(*a*) concentration of B hormone in the circulation of a dark fish,

(*b*) concentration of B hormone in the pituitary of a dark fish,

(*c*) total blood volume,

indicate that the distribution of B between pituitary and circulation in a dark anim is of the order pituitary 1, circulation 10.

4. Caustic soda treated extracts of posterior lobe pituitary which evoke the sam rise of melanophore index as untreated extracts do not maintain the expand condition of the melanophores for the same period. The factors involved a discussed.

Thanks are due to the Carnegie Trust for a grant to one of us (H.W.) towar the costs of this investigation.

REFERENCES

ABRAMOWITZ, A. A. (1937). *Biol. Bull. Wood's Hole*, **73**, 134.
BÖTTGER, G. (1937). *Z. ges. exp. Med.* **101**, 54.
BURN, J. H. (1931). *Quart. J. Pharmacol.* **4**, 517.
CREASER, C. W. & GORBMAN, A. (1939). *Quart. Rev. Biol.* **14**, 311.
FENN, W. (1924). *J. Physiol.* **59**, 35.
HELLER, H. (1939). *J. Physiol.* **96**, 337.
—— (1940). *J. Physiol.* **98**, 405.
HOGBEN, L. (1924). *The Pigmentary Effector System.* London: Oliver and Boyd.
—— (1927). *The Comparative Physiology of Internal Secretion.* Cambridge.
HOGBEN, L. & DE BEER, G. R. (1925). *Quart. J. exp. Physiol.* **15**, 163.
HOGBEN, L. & GORDON, C. (1930). *J. exp. Biol.* **7**, 286.
HOGBEN, L. & LANDGREBE, F. W. (1940). *Proc. Roy. Soc.* B, **128**, 317.
HOGBEN, L., SCHLAPP, W. & M'DONALD, A. D. (1924). *Quart. J. exp. Physiol.* **14**, 301.
HOGBEN, L. & SLOME, D. (1931). *Proc. Roy. Soc.* B, **108**, 10.
—— —— (1936). *Proc. Roy. Soc.* B, **120**, 158.
HOGBEN, L. & WINTON, F. (1923). *Proc. Roy. Soc.* B. **94**, 151.
JORES, A. (1933). *Z. ges. exp. Med.* **87**, 266.
—— (1934). *Klin. Wschr.* **13**, 1269.
JORES, A. & HOELTZ, K. (1936). *Z. vergl. Physiol.* **23**, 571.
JORES, A. & LENSSEN, E. W. (1933). *Endocrinologie*, **12**, 90.
JORES, A. & WILL, H. (1934). *Z. ges. exp. Med.* **94**, 389.
KLEINHOLZ, L. H. (1938). *J. exp. Biol.* **15**, 492.
KLEINHOLZ, L. H. & RAHN, W. (1940). *Anat. Rec.* **76**, 157.
KOLLER, G. & RODEWALD, W. (1933). *Pflüg. Arch. ges. Physiol.* **232**, 183.
LIEBEN, S. (1906). *Zbl. Physiol.* **20**, 108.
LUNDSTROM, H. M. & BARD, P. (1932). *Biol. Bull. Wood's Hole*, **62**, 1.
MASSELIN, J. N. (1939). *Rev. Soc. argent. Biol.* **15**, 28.

L, R. M. (1940). *J. exp. Biol.* **17**, 74.
ERHAGE, K. H. (1932). *Z. mikr. Anat. Forsch.* **30**, 551.
KER, G. H. (1936). *Colour Change of Animals in Relation to Nervous Activity*. Philadelphia.
— (1938). *J. exp. Biol.* **15**, 48.
KER, G. H. & SCATTERTY, L. E. (1937). *J. cell. comp. Physiol.* **9**, 3.
EWALD, W. (1935). *Z. vergl. Physiol.* **21**, 767.
D, A. (1935). *Biol. Rev.* **10**, 361.
TH, H. G. (1938). *Proc. Roy. Soc.* B. **125**, 250.
ETH, R. A. & BARBOUR, H. G. (1917). *J. Pharmacol.* **9**, 431.
HLE, R. L. (1936). *J. Pharmacol.* **57**, 1.
— (1938). *Ergeb. der Vit. und Hormonforschung*, **1**, 114.
RING, H. (1936*a*). *Proc. Lpool Biol. Soc.* **49**, 17.
— (1936*b*). *Proc. Lpool Biol. Soc.* **49**, 65.
— (1938). *Proc. Roy. Soc.* B, **125**, 264.
— (1940). *Proc. Roy. Soc.* B, **128**, 343.
NG, J. Z. (1935). *J. exp. Biol.* **12**, 254.